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(54) Canine coronavirus subunit vaccine.

(57) The invention is related to a nucleic acid sequence encoding a Canine coronavirus (CCV) spike protein. Such a protein can be used for the immunization of dogs against CCV infection. The nucleic acid sequence encoding the CCV spike protein can be applied for the preparation of the spike protein by means of genetic engineering techniques or can be applied for the preparation of vector vaccines.

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The present invention is concerned with a nucleic acid sequence encoding a CCV spike protein, a recombinant vector or recombinant vector virus comprising such a nucleic acid sequence, a host cell transformed with such a recombinant vector or infected with the recombinant vector virus, as well as a vaccine against CCV infection in dogs.

Canine coronavirus (CCV) is a member of the distinct viral family of Coronavirus. Viruses belonging to this genus are known to infect a variety of animal species including man. They cause diverse diseases, such as gastro-enteritis (in swine, turkeys, mice, calves, dogs, cats and man), salivary gland infection (in rodents), respiratory disease (in man, swine, avians and dogs) and encephalitis (in young swine).

CCV was first isolated from military dogs in Germany in 1971 and has found to be highly contagious and it spreads rapidly among susceptible dogs. Usually, the CCV is ingested on materials contaminated by infectious feces. Oral infection leads to viral replication in epithelial cells of the small intestine and CCV has also been found in the intestinal lymph nodes.

The signs of the disease can develop 1-3 days following infection and include vomiting, diarrhoea, anorexia, depression and dehydration. The persistence and severity of signs is often related to stress and the presence of other viruses, parasites or bacteria. Whereas the enteric symptoms are dominant, respiratory signs including nasal and ocular discharge have also been reported.

Dogs are the only known host of the CCV. Although CCV inoculation of cats and pigs results in infection, no clinical disease will be caused by CCV in these species. There is no evidence that humans, cattle and mice are susceptible to CCV.

Cross protection studies have shown that the Coronaviruses induce little or no immunity to each other. For example, experimental infection of dogs with transmissible gastro-enteritis virus (TGEV) of pigs or feline infectious peritonitis virus (FIPV) of cat does not protect them against the effects of a subsequent CCV infection.

Coronaviruses consist of a group of enveloped viruses containing a genome consisting of a single-stranded RNA of about 30 kb. This genome encodes inter alia three important structural proteins: a spike protein (S), a membrane protein (M) and a nucleocapsid protein (N). The glycosylated spike protein S_o is cleaved to form S_1 and S_2 in some coronaviruses. Two or three copies of each of S_1 and S_2 form a characteristic CCV surface structure, the spike or peplomer. The spike protein and its constituent polypeptides thereof play an important role in inducing a virus neutralizing immune response in infected animals.

Conventional CCV vaccines comprise chemically inactivated virus vaccines or modified live-virus vaccines. However, inactivated vaccines require additional immunizations, disadvantageously contain adjuvants and are expensive to produce. Further, some infectious virus particles may survive the inactivation process and may cause disease after administration to the animal.

In general, attenuated live virus vaccines are preferred because they evoke an immune response often based on both humoral and cellular reactions. Up to now, such vaccines based on CCV strains can only be prepared by serial passage of virulent strains in tissue culture. However, because of this treatment uncontrolled mutations are introduced into the viral genome, resulting in a population of virus particles heterogeneous in their virulence and immunizing properties. In addition it is well known that such traditional attenuated live virus vaccines can revert to virulence resulting in disease of the inoculated animals and the possible spread of the pathogen to other animals.

Improved vaccines might be constructed, based on recombinant DNA technology, which only contain the necessary and relevant CCV immunogenic material capable of eliciting an immune response against the CCV pathogens, or which contain the genetic information encoding said material, and do not display above-mentioned disadvantages of the live or inactivated vaccines.

According to the present invention, an isolated and purified nucleic acid sequence encoding a polypeptide having one or more immunogenic determinants of a CCV spike protein is provided which can be applied for the preparation of a vaccine for the immunization of dogs against CCV infection.

"Nucleic acid sequence" as used herein refers to a polymeric form of nucleotides of any length, both to ribonucleic acid sequences and to deoxy ribonucleic acid sequences. In principle, this term refers to the primary structure of the molecule. Thus, this term includes double and single stranded DNA, as well as double and single stranded RNA, and modifications thereof.

In general, the term "polypeptide" refers to a molecular chain of amino acids with a biological activity, does not refer to a specific length of the product and if required can be modified in vivo or in vitro, for example by glycosylation, amidation, carboxylation or phosphorylation; thus inter alia, peptides, oligopeptides and proteins are included.

The term "polypeptide having one or more immunogenic determinants of a CCV spike protein" refers to a polypeptide having one or more epitopes capable of eliciting a protective immune response in a dog against CCV infection or disease.

In particular, the present invention provides a nucleic acid sequence encoding a polypeptide having one or more immunogenic determinants of the CCV spike protein which has an amino acid sequence shown in SEQ ID NO: 2, 4 or 6.

Also included within the scope of the present invention are nucleic acid sequences encoding a functional variant of the polypeptide shown in SEQ ID NO: 2, 4 or 6. These functional variants are polypeptides having an amino acid sequence derived from the amino acid sequence specifically disclosed in SEQ ID NO: 2, 4 or 6 but retain one or more immunogenic determinants of a CCV spike protein, i.e. said variants having one or more epitopes capable of eliciting a protective immune response in a dog against CCV infection or disease.

It will be understood that for the particular polypeptide embraced herein, derived from the CCV-6, Insavc-1 or Liverpool C54 strain, natural variations can exist between individual viruses or strains of canine coronaviruses. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. Amino acid substitutions from which can be expected that they do not essentially alter biological and immunological activities, have been described. Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, Ile/Val (see Dayhof, M.D., Atlas of protein sequence and structure, Nat. Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3). Based on this information Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science 227, 1435-1441, 1985) and determining the functional similarity between homologous polypeptides. Nucleic acid sequences encoding such homologous functional variants are included within the scope of this invention. Moreover, the potential exists to use recombinant DNA technology for the preparation of nucleic acid sequences encoding these various functional variants.

Nucleic acid sequences according to the invention may be derived from isolates of CCV strains such as CCV-6, Insavc-1 (EP 396,193), CCV 1-71 (ATCC VR-809) or CCV TN449 (ATCC VR-2068).

In another aspect of the invention nucleic acid sequences described above are provided which can be used for the preparation of a vaccine to protect cats against FIPV infection.

The information provided in SEQ ID NO: 1-6 allows a person skilled in the art to isolate and identify the nucleic acid sequences encoding the various functional variant polypeptidec mentioned-above having corresponding immunological characteristics with the CCV spike protein specifically disclosed herein. The generally applied Southern blotting technique or colony hybridization can be used for that purpose (Experiments in Molecular Biology, ed. R.J. Slater, Clifton, U.S.A., 1986; Singer-Sam, J. et al., Proc. Natl. Acad. Sci. 80, 802-806, 1983; Maniatis T. et al., Molecular Cloning, A laboratory Manual, second edition, Cold Spring Harbor Laboratory Press, USA, 1989). For example, RNA or cDNA derived from a specific CCV strain is electrophoresed and transferred, or "blotted" thereafter onto a piece of nitrocellulose filter. It is now possible to identify CCV spike protein nucleic acid sequences on the filter by hybridization to a defined labeled DNA fragment or "probe", i.e. a (synthetic) poly- or oligonucleotide sequence fragment of the nucleic acid sequence shown in SEQ ID NO: 1, 3 or 5 which under specific conditions of salt concentration and temperature hybridizes to the homologous nucleic acid sequences present on the filter. After washing the filter, hybridized material may be detected by autoradiography. The corresponding DNA fragment can now be eluted from the agarose gel and used to direct the synthesis of a functional variant of the polypeptide disclosed in SEQ ID NO: 2, 4 or 6.

Therefore, a preferred functional variant according to the invention is a polypeptide comprising one or more immunogenic determinants of a CCV spike protein and is encoded by a nucleic acid sequence which hybridizes to the DNA sequence shown in SEQ ID NO: 1, 3 or 5.

In another way CCV cDNA may be cloned into a $_{\kappa}$gt11 phage as described by Huynh et al. (In: D. Glover (ed.), DNA Cloning: A Practical Approach, IRL Press Oxford, 49-78, 1985) and expressed in a bacterial host. Recombinant phages can then be screened with polyclonal serum raised against the purified CCV spike protein disclosed in SEQ ID NO: 2, 4 or 6 determining the presence of corresponding immunological regions of the variant polypeptide. The production of the polyclonal serum to be used herein elicited against the CCV spike protein is described below.

As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in an other codon but still coding for the same amino acid, e.g. the codon for the amino acid glutamic acid is both GAT and GAA. Consequently, it is clear that for the expression of a polypeptide with the amino acid sequence shown in SEQ ID NO: 2, 4 or 6 use can be made of a derivate nucleic acid sequence with such an alternative codon composition different from the nucleic acid sequence shown in SEQ ID NO: 1, 3 or 5, respectively.

Furthermore, also fragments of the nucleic acid sequences encoding the specifically disclosed CCV

spike protein or functional variants thereof as mentioned above are included in the present invention.

The term "fragment" as used herein means a DNA or amino acid sequence comprising a subsequence of the nucleic acid sequence or polypeptide of the invention. Said fragment is or encodes a polypeptide having one or more immunogenic determinants of a CCV spike protein, i.e. has one or more epitopes which are capable of eliciting a protective immune response in a dog. Methods for determining usable polypeptide fragments are outlined below. Fragments can inter alia be produced by enzymatic cleavage of precursor molecules, using restriction endonucleases for the DNA and proteases for the polypeptides. Other methods include chemical synthesis of the fragments or the expression of polypeptide fragments by DNA fragments.

Typical sequences encoding the CCV spike protein precursor are shown in SEQ ID NO: 1, 3 and 5. These cDNA sequences are about 4328, 4352 and 4358 nucleotides in length, respectively, and encode a polypeptide of 1443, 1451 and 1453 amino acids, respectively.

A preferred nucleic acid sequence according to the invention is characterized in that said sequence contains at least part of the DNA sequence disclosed in SEQ ID NO: 1, 3 or 5.

A nucleic acid sequence according to the invention may be isolated from a particular CCV strain and multiplied by recombinant DNA techniques including polymerase chain reaction (PCR) technology or may be chemically synthesized in vitro by techniques known in the art.

All modifications resulting in the above-mentioned functional variants of the specifically exemplified polypeptide are included within the scope of the present invention for as long as the resulting polypeptides retain one or more immunogenic determinants of a CCV spike protein.

A nucleic acid sequence according to the present invention can be ligated to various replication effecting DNA sequences with which it is not associated or linked in nature resulting in a so called recombinant vector molecule which can be used for the transformation of a suitable host. Useful recombinant vector molecules, are preferably derived from, for example plasmids, bacteriophages, cosmids or viruses.

Specific vectors or cloning vehicles which can be used to clone nucleic acid sequences according to the invention are known in the art and include inter alia plasmid vectors such as pBR322, the various pUC, pGEM and Bluescript plasmids, bacteriophages, e.g. $_{K}$gt-Wes,Charon 28 and the M13 derived phages or viral vectors such as SV40, adenovirus or polyoma virus (see also Rodriquez, R.L. and D.T. Denhardt, ed., Vectors: A survey of molecular cloning vectors and their uses, Butterworths, 1988; Lenstra, J.A. et al., Arch. Virol. 110, 1-24, 1990). The methods to be used for the construction of a recombinant vector molecule according to the invention are known to those of ordinary skill in the art and are inter alia set forth in Maniatis, T. et al. (Molecular Cloning A Laboratory Manual, second edition; Cold Spring Harbor Laboratory, 1989).

For example, the insertion of the nucleic acid sequence according to the invention into a cloning vector can easily be achieved when both the genes and the desired cloning vehicle have been cut with the same restriction enzyme(s) as complementary DNA termini are thereby produced.

Alternatively, it may be necessary to modify the restriction sites that are produced into blunt ends either by digesting the single-stranded DNA or by filling in the single-stranded termini with an appropriate DNA polymerase. Subsequently, blunt end ligation with an enzyme such as T4 DNA ligase may be carried out.

If desired, any restriction site may be produced by ligating linkers onto the DNA termini. Such linkers may comprise specific oligonucleotide sequences that encode restriction site sequences. The restriction enzyme cleaved vector and nucleic acid sequence may also be modified by homopolymeric tailing.

"Transformation", as used herein, refers to the introduction of a heterologous nucleic acid sequence into a host cell, irrespective of the method used, for example direct uptake or transduction. The heterologous nucleic acid sequence may be maintained through autonomous replication or alternatively, may be integrated into the host genome. If desired, the recombinant vector molecules are provided with appropriate control sequences compatible with the designated host which can regulate the expression of the inserted nucleic acid sequence. In addition to microorganisms, culture of cells derived from multicellular organisms may also be used as hosts.

The recombinant vector molecules according to the invention preferably contain one or more marker activities that may be used to select for desired transformants, such as ampicillin and tetracycline resistance in pBR322, ampicillin resistance and β-galactosidase activity in pUC8.

A suitable host cell is a microorganism or cell which can be transformed by a nucleic acid sequence encoding a polypeptide or by a recombinant vector molecule comprising such a nucleic acid sequence and which can if desired be used to express said polypeptide encoded by said nucleic acid sequence. The host cell can be of procaryotic origin, e.g. bacteria such as Escherichia coli, Bacillus subtilis and Pseudomonas species; or of eucaryotic origin such as yeasts, e.g. Saccharomyces cerevisiae or higher eucaryotic cells such as insect, plant or mammalian cells, including HeLa cells and Chinese hamster ovary (CHO) cells.

Insect cells include the Sf9 cell line of Spodoptera frugiperda (Luckow et al., Bio-technology 6, 47-55, 1988). Information with respect to the cloning and expression of the nucleic acid sequence of the present invention in eucaryotic cloning systems can be found in Esser, K. et al. (Plasmids of Eukaryotes, Springer-Verlag, 1986).

In general, prokaryotes are preferred for the construction of the recombinant vector molecules useful in the invention. For example E.coli K12 strains are particularly useful such as DH5α or JM101.

For expression nucleic acid sequences of the present invention are introduced into an expression vector, i.e. said sequences are operably linked to expression control sequences. Such control sequences may comprise promoters, enhancers, operators, inducers, ribosome binding sites etc. Therefore, the present invention provides a recombinant vector molecule comprising a nucleic acid sequence encoding the CCV spike protein operably linked to expression control sequences, capable of expressing the DNA sequences contained therein in (a) transformed host cell(s).

It should, of course, be understood that the nucleotide sequences inserted at the selected site of the cloning vector may include nucleotides which are not part of the actual structural gene for the desired polypeptide or may include only a fragment of the complete structural gene for the desired protein as long as transformed host will produce a polypeptide having at least one or more immunogenic determinants of a CCV spike protein.

When the host cells are bacteria, illustrative useful expression control sequences include the Trp promoter and operator (Goeddel, et al., Nucl. Acids Res. 8, 4057, 1980); the lac promoter and operator (Chang, et al., Nature 275, 615, 1978); the outer membrane protein promoter (Nakamura, K. and Inouge, M., EMBO J. 1, 771-775, 1982); the bacteriophage $_k$ promoters and operators (Remaut, E. et al., Nucl. Acids Res. 11, 4677-4688, 1983); the α-amylase (B. subtilis) promoter and operator, termination sequence and other expression enhancement and control sequences compatible with the selected host cell. When the host cell is yeast, illustrative useful expression control sequences include, e.g., α-mating factor. For insect cells the polyhedrin or p10 promoters of baculoviruses can be used (Smith, G.E. et al., Mol. Cell. Biol. 3, 2156-65, 1983). When the host cell is of mammalian origin illustrative useful expression control sequences include, e.g., the SV-40 promoter (Berman, P.W. et al., Science 222, 524-527, 1983) or, e.g. the metallothionein promoter (Brinster, R.L., Nature 296, 39-42, 1982) or a heat shock promoter (Voellmy et al., Proc. Natl. Acad. Sci. USA 82, 4949-53, 1985). For maximizing gene expression, see also Roberts and Lauer (Methods in Enzymology 68, 473, 1979).

Therefore, the invention also comprises (a) host cell(s) transformed with a nucleic acid sequence or recombinant expression vector molecule described above, capable of producing the CCV spike protein by expression of the nucleic acid sequence.

The present invention also provides a process for the preparation of a purified polypeptide displaying immunological characteristics of a CCV spike protein, i.e. the polypeptide has one or more immunogenic determinants of a CCV spike protein, essentially free from whole viruses or other protein with which it is ordinarily associated.

More particularly, the invention provides a process for the preparation of a polypeptide comprising at least part of the amino acid sequence shown in SEQ ID NO: 2, 4 or 6 or a functional variant thereof.

In addition a polypeptide substantially comprising an immunogenic fragment of the CCV spike protein which can be used for immunization of dogs against CCV infection or diagnostic purposes, is prepared in the present invention. Various methods are known for detecting such usable immunogenic fragments within an amino acid sequence.

Suitable immunochemically active polypeptide fragments of a polypeptide according to the invention containing (an) epitope(s) can be found by means of the method described in Patent Application WO 86/06487, Geysen, H.M. et al. (Prod. Natl. Acad. Sci. 81, 3998-4002, 1984), Geysen, H.M. et al. (J. Immunol. Meth. 102, 259-274, 1987) based on the so-called pep-scan method, wherein a series of partially overlapping peptides corresponding with partial sequences of the complete polypeptide under consideration, are synthesized and their reactivity with antibodies is investigated.

In addition, a number of regions of the polypeptide, with the stated amino acid sequence, can be designated epitopes on the basis of theoretical considerations and structural agreement with epitopes which are now known. The determination of these regions is based on a combination of the hydrophilicity criteria according to Hopp and Woods (Proc. Natl. Acad. Sci. 78, 3824-3828, 1981) and the secondary structure aspects according to Chou and Fasman (Advances in Enzymology 47, 45-148, 1987).

T-cell epitopes which may be necessary can likewise be derived on theoretical grounds, e.g. with the aid of Berzofsky's amphiphilicity criterion (Science 235, 1059-62, 1987).

In another embodiment of the invention a polypeptide having an amino acid sequence encoded by a nucleic acid sequence mentioned above is used.

Immunization of dogs against CCV infection can, for example be achieved by administering to the animals a polypeptide prepared according to the process mentioned above in an immunologically relevant context as a so-called subunit vaccine. The subunit vaccine according to the invention may comprise a polypeptide in a pure form, optionally in the presence of a pharmaceutically acceptable carrier. The polypeptide can optionally be covalently bound to a non-related protein, which, for example can be of advantage in the purification of the fusion product. Examples are β-galactosidase, protein A, prochymosine, blood clotting factor Xa, etc.

In some cases the ability to raise neutralizing antibodies against these polypeptides per se may be low. Small fragments are preferably conjugated to carrier molecules in order to raise their immunogenicity. Suitable carriers for this purpose are macromolecules, such as natural polymers (proteins like key hole limpet hemocyanin, albumin, toxins), synthetic polymers like polyamino acids (polylysine, polyalanine), or micelles of amphiphilic compounds like saponins. Alternatively these fragments may be provided as polymers thereof, preferably linear polymers.

Polypeptides to be used in such subunit vaccines can be prepared by methods known in the art, e.g. by isolating said polypeptides from CCV, by recombinant DNA techniques or by chemical synthesis.

If required these polypeptides to be used in a vaccine can be modified in vitro or in vivo, for example by glycosylation, amidation, carboxylation or phosphorylation.

An alternative to subunit vaccines are live vector vaccines. A nucleic acid sequence according to the invention is introduced by recombinant DNA techniques into a micro-organism (e.g. a bacterium or virus) in such a way that the recombinant micro-organism is still able to replicate thereby expressing a polypeptide coded by the inserted nucleic acid sequence and eliciting an immune response in the infected host animal.

A preferred embodiment of the present invention is a recombinant vector virus comprising a heterologous nucleic acid sequence described above, capable of expressing the DNA sequence in (a) host cell(s) or host animal infected with the recombinant vector virus. The term "heterologous" indicates that the nucleic acid sequence according to the invention is not normally present in nature in the vector virus.

Furthermore, the invention also comprises (a) host cell(s) or cell culture infected with the recombinant vector virus, capable of producing the CCV protein by expression of the nucleic acid sequence.

For example the well known technique of in vivo homologous recombination can be used to introduce a heterologous nucleic acid sequence, e.g. a nucleic acid sequence according to the invention into the genome of the vector virus.

First, a DNA fragment corresponding with an insertion region of the vector genome, i.e. a region which can be used for the incorporation of a heterologous sequence without disrupting essential functions of the vector such as those necessary for infection or replication, is inserted into a cloning vector according to standard recDNA techniques. Insertion-regions have been reported for a large number of micro-organisms (e.g. EP 80,806, EP 110,385, EP 83,286, US 4,769,330 and US 4,722,848).

Second, if desired, a deletion can be introduced into the insertion region present in the recombinant vector molecule obtained from the first step. This can be achieved for example by appropriate exonuclease III digestion or restriction enzyme treatment of the recombinant vector molecule from the first step.

Third, the heterologous nucleic acid sequence is inserted into the insertion-region present in the recombinant vector molecule of the first step or in place of the DNA deleted from said recombinant vector molecule. The insertion region DNA sequence should be of appropriate length as to allow homologous recombination with the vector genome to occur. Thereafter, suitable cells can be infected with wild-type vector virus or transformed with vector genomic DNA in the presence of the recombinant vector molecule containing the insertion flanked by appropriate vector DNA sequences whereby recombination occurs between the corresponding regions in the recombinant vector molecule and the vector genome. Recombinant vector progeny can now be produced in cell culture and can be selected for example genotypically or phenotypically, e.g. by hybridization, detecting enzyme activity encoded by a gene co-integrated along with the heterologous nucleic acid sequence, or detecting the antigenic heterologous polypeptide expressed by the recombinant vector immunologically.

Next, this recombinant micro-organism can be administered to the dogs for immunization whereafter it maintains itself for some time, or even replicates in the body of the inoculated animal, expressing in vivo a polypeptide coded for by the inserted nucleic acid sequence according to the invention resulting in the stimulation of the immune system of the inoculated animal. Suitable vectors for the incorporation of a nucleic acid sequence according to the invention can be derived from viruses such as pox viruses, e.g. vaccinia virus (EP 110,385, EP 83,286, US 4,769,330 and US 4,722,848), herpes viruses such as Feline Herpes virus, (canine) adeno virus (WO 91/11525) or influenza virus, or bacteria such as E. coli or specific Salmonella species. With recombinant microorganisms of this type, the polypeptide synthesized in the host can be exposed as a cell surface antigen. In this context fusion of the said polypeptide with OMP proteins,

or pilus proteins of for example E. coli or synthetic provision of signal and anchor sequences which are recognized by the organism are conceivable. It is also possible that the said immunogenic polypeptide, if desired as part of a larger whole, is released inside the animal to be immunized. In all of these cases it is also possible that one or more immunogenic products will find expression which generate protection against various pathogens and/or against various antigens of a given pathogen.

A vaccine according to the invention can be prepared by culturing a host cell infected with a recombinant vector virus comprising a nucleic acid sequence according to the invention, whereafter virus containing cells and/or recombinant vector viruses grown in the cells can be collected, optionally in a pure form, and formed to a vaccine optionally in a lyophilized form.

Host cells transformed with a recombinant vector molecule according to the invention can also be cultured under conditions which are favourable for the expression of a polypeptide coded by said nucleic acid sequence. Vaccines may be prepared using samples of the crude culture, host cell lysates or host cell extracts, although in another embodiment more purified polypeptides according to the invention are formed to a vaccine, depending on its intended use. In order to purify the polypeptides produced, host cells transformed with a recombinant vector according to the invention are cultured in an adequate volume and the polypeptides produced are isolated from such cells or from the medium if the protein is excreted. Polypeptides excreted into the medium can be isolated and purified by standard techniques, e.g. salt fractionation, centrifugation, ultrafiltration, chromatography, gel filtration or immuno affinity chromatography, whereas intra cellular polypeptides can be isolated by first collecting said cells, disrupting the cells, for example by sonication or by other mechanically disruptive means such as French press followed by separation of the polypeptides from the other intra cellular components and forming the polypeptides to a vaccine. Cell disruption could also be accomplished by chemical (e.g. EDTA treatment) or enzymatic means such as lysozyme digestion.

The vaccine according to the invention can be administered in a convential active immunization scheme: single or repeated administration in a manner compatible with the dosage formulation and in such amount as will be prophylactically and/or therapeutically effective and immunogenic. i.e. the amount of immunizing antigen or recombinant micro-organism capable of expressing said antigen that will induce immunity in a dog against challenge by a virulent CCV. Immunity is defined as the induction of a significant level of protection in a population of dogs after vaccination compared to an unvaccinated group.

For live viral vector vaccines the dose rate per dog may range from 10^5 - 10^8 pfu.

A typical subunit vaccine according to the invention comprises 10 μg - 1 mg of the polypeptide according to the invention.

The administration of the vaccine can be done, e.g. intradermally, subcutaneously, intramuscularly, intraperitonially, intravenously, orally or intranasally.

Additionally the vaccine may also contain an aqueous medium or a water containing suspension, often mixed with other constituents, e.g. in order to increase the activity and/or shelf life. These constituents may be salts, pH buffers, stabilizers (such as skimmed milk or casein hydrolysate), emulsifiers adjuvants to improve the immune response (e.g. oils, muramyl dipeptide, aluminiumhydroxide, saponin, polyanions and amphipatic substances) and preservatives.

It is clear that a vaccine according to the invention may also contain immunogens related to other pathogens of dogs or may contain nucleic acid sequences encoding these immunogens, like antigens of Canine parvovirus (CPV), Canine Distemper virus, Canine Adenovirus I, Canine Adenovirus II, Canine Parainfluenza virus, Canine Rotavirus or Leptospira canicola to produce a multivalent vaccine.

Example 1

A.

1. Preparation of genomic viral RNA of CCV-6 and Liverpool C54 strain

Confluent A-72 cells grown in plastic tissue culture flasks using the Wellcome modification of minimal Eagle's medium (MEM) and 10% foetal bovine serum were infected with CCV (NVSL Challenge virus CCV-6 from the National Veterinary Service Laboratory, PO Box 844, Ames, Iowa 50010, USA) at a multiplicity of infection (MOI) of approximately 0.1. After 24 h the culture supernatant was harvested, chilled to 4 °C and cell debris removed by centrifugation at 3000 x g for 15 min. Virus was pelletted from the supernatant at 53.000 x g for 2 h in a Beckman type 19 rotor. The pellet was resuspended in 5 ml of TNE (10 mM Tris-Cl, 100 mM NaCl, 1 mM EDTA, pH 7.5) using a Dounce homogeniser and layered onto a 32 ml linear 20-60% gradient of sucrose in TNE. The virus was banded isopycnically by overnight centrifugation at 100.000 x g in a Beckman SW28 rotor. The gradient was fractionated and the A_{280}'s and densities of the fractions determined. A peak was identified at the characteristic

density of 1.18 g/cc. The peak fractions were pooled, diluted in TNE and the putative virus pelletted by centrifugation at 100.000 x g for 2 h in the Beckman SW28 rotor. RNA was isolated from the virus pellet using two approaches:

A. The pellet was resuspended in 0.1 M Tris-Cl pH 8.0 containing 0.1% SDS and digested for 3 h at 50 °C with 20 µg/ml of proteinase K. The mixture was deproteinised using phenol:chloroform:isoamyl alcohol (50:49:1) saturated with TE (10 mM Tris-Cl 1 mM EDTA) and the nucleic acid recovered by precipitation with 2.5 volumes of ethanol/0.3 M sodium acetate pH 5.2. The preparation was analysed on a Tris-borate EDTA 1% agarose gel containing 0.1% SDS; a high molecular weight RNA band was identified with the characteristic mobility of coronavirus genomic RNA.

B. The virus pellet was homogenised in 6 M guanidinium isothiocyanate/5 mM sodium citrate (pH 7.0)/0.1 M mercaptoethanol/0.5% N-lauroyl sarcosinate and 1 g/ml of CsCl added to each 2.5 ml of the homogenate. The mixture was then layered onto a 5.7 M CsCl/0.1 M EDTA pad and centrifuged at 108.000 x g for 12 h at 20 °C. The pellet of RNA was dissolved in TE containing 0.1% SDS. The preparation was analysed as described above.

2. cDNA cloning of CCV genomic RNA

First strand synthesis from 2 µg of CCV genomic RNA prepared as described in 1A above was primed with 1 ng of a specific oligonucleotide (5' TTTTCAAATTGTCTTCTACTT 3') using 40 units of AMV reverse transcriptase in a reaction volume of 25 µl containing 20 mM Tris-Cl (pH 8.3 at 42 °C), 0.14 M KCl, 10 mM $MgCl_2$, 1 mM dNTP's, 4 mM dithiothreitol, 25 units of human placental ribonuclease inhibitor. The reaction mixture was incubated for 1 h at 42 °C. Second strand synthesis was achieved by addition of 46 µl of a reaction mixture containing 7.6 mM $MgCl_2$, 0.109 M Tris-Cl pH 7.4, 16.3 mM $(NH_4)_2SO_4$, 1000 units/ml RNaseH, 10.000 units/ml E. coli DNA polymerase 1 to the first strand reaction and incubation at 12 °C for 1 h followed by incubation at 22 °C for a further 1 h. The reaction products were deproteinised by two extractions with phenol:chloroform:isoamyl alcohol (50:49:1) saturated with TE and precipitated with 2 volumes of ethanol/0.3 M sodium acetate pH 5.2. The cDNA was tailed with C residues using terminal deoxynucleotidyl transferase using the buffer and conditions supplied by the manufacturer (Bethesda Research Laboratories, Gaithersburg, Maryland 20877, USA). It was then size fractionated on a 2 ml Sephacryl S-1000 column and cDNA of size greater than 500 base pairs pooled, ethanol precipitated and dissolved in TE. 50 ng of this cDNA was annealed with 250 ng of dG-tailed PstI cut pUC119. The mixture was transformed into E. coli TG-1. Ampicillin resistant transformants were picked and screened for CCV cDNA inserts using a cDNA probe produced by random priming of reverse transcription from CCV genomic RNA. Positive colonies were screened for size of cDNA inserts by PstI digestion of mini-prep DNA. The relationships between inserts were established by restriction enzyme analysis. The clone pBHI was selected for sequence analysis. The size of the pBHI insert (4.0 kb) was insufficient to cover the complete CCV spike coding region and a further round of cDNA synthesis and cloning was carried out using another specific primer (5' CTAGGTAGTAACAC 3'). The RNA used was isolated as described in 1B above. cDNA synthesis was achieved using a Boehringer Mannheim (Boehringer Mannheim UK, Bell Lane, Lewes, East Sussex BN7 1LG) cDNA synthesis kit according to the manufacturer's instructions. In summary first strand synthesis was again achieved using AMV reverse transcriptase, second strand synthesis by the action of E. coli DNA polymerase 1 and RNaseH. The cDNA was blunt ended by the action of T4 DNA polymerase. The cDNA was ligated into SmaI-cut phosphatased pUC18 using T4 DNA ligase and the DNA transformed into E. coli TG1. Ampicillin resistant clones were initially screened for inserts using blue/white selection on X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) plates. White colonies were picked and screened for the presence of CCV cDNA inserts as described above. Clone pBH2 (size 2.8 kb) was selected for sequence analysis. The same strategy as outlined in Example 1.1.B. and 1.2. for CCV strain CCV6 was carried out for the isolation of the spike gene of the CCV C54 strain. Three overlapping clones, pBH3, pBH4 and pBH11 covered the spike gene to the blunt end.

3. DNA sequencing The cDNA inserts from clones pBH1, pBH2, and pBH3, pBH4 and pBH11 were sequenced using the Sanger dideoxy chain termination method. This shotgun approach was supplemented as necessary with sequencing from specific oligonucleotide primers on double stranded plasmid DNA templates. For the shotgun analysis insert DNA was excised from the vector sequences, circularised, sonicated, size selected on agarose gels and cloned into SmaI-cut, phosphatased M13mp8. Shotgun sequence data were assembled using the DBUTIL and DBAUTO programs of Staden and analysed using the ANALYSEQ/NIP packages of Staden. A VAX 8350 and micro VAX 3100 (Digital Equipment Corporation) were used. The sequence data are presented in SEQ ID NO: 1

and 5.

B.

1. Preparation of genomic viral RNA of Insavc-1 strain

Confluent A-72 cells grown in plastic tissue culture flasks using the Wellcome modification of minimal Eagle's medium (MEM) and 10% F.C.S. were infected with CCV strain Insavc-1 (Bert) (Intervet Labs.) at a m.o.i. of approximately 0.1. After 48 hours the culture supernatant was harvested, chilled to 4 ° and cell debris removed by centrifugation at 3000xg for 15 minutes. Virus was pelleted from the supernatant at 53000xg for 2 hrs in a Beckman type 19 rotor. The virus pellet was homogenized in 3.5 mls of 6M guanidinium isothiocyanate/5mM sodium citrate (pH 7.0), 0.1M mercaptoethanol, 0.5% N-lauroyl sarcosinate.

The homogenate was layered onto a 5.7 M CsCl pad (1 ml) and centrifuged at 108000 g for 18 hours at 18 °C. The pellet of RNA was dissolved in TE containing 0.1% SDS, then precipitated twice with 2.5 volumes of ethanol/0.3M NaOAc pH 5.2. The preparation was analysed as a Tris-borate EDTA 1% agarose gel containing 0.1% SDS, high molecular weight RNA band was identified with the characteristic mobility of coronavirus genomic RNA.

2. cDNA and PCR cloning of CCV genomic RNA

First and second strand synthesis from 2 µg of CCV genomic RNA prepared as aforementioned was primed with oligo dT and random pentanucleotides from the Boehringer cDNA synthesis kit under the conditions specified by the manufacturers protocol.

The resultant blunt ended cDNA produced from this reaction was ligated into Sma1-cut phosphatased pUC 119 using T4 DNA ligase and the DNA transformed into E. coli TG-1. Ampicillin resistant clones were initially screened for inserts using blue/white selection on x-gal (5-bromo-4-chloro-3-indetyl-B-D-galaclopyramoside) plates. White colonies were picked and screened for the presence of CCV cDNA inserts using randomly primed CCV RNA as a probe. Five positive clones were identified.

Plasmid pBH6 was generated using the polymerase chain reaction (PCR). Sequence information from the ends of pBH5 and pBH7 allowed the design of primers BH7 and BH8. A Not 1 site was incorporated into the oligo's to facilitate cloning. Briefly, approximately 1 ng of first-strand reaction as described previously was deproteinized by two extractions with phenol:chloroform:Isoramyl alcohol (50:49:1) saturated with TE, passed down a G50 spin column and precipitated with two volumes of ethanol/0.3 M sodium acetate pH 5.2. The DNA:RNA hybrids were resuspended in 15 µl TE. The PCR reaction was carried out with the Techne programmable Dri-block PHC-1.

The generated fragment was phenol/chloroform ethanol precipitated as before and resuspended in 20 µl of TE. The DNA was cleaved with Not 1 under conditions recommended by the enzyme manufacturer, and gel eluted. The Not 1 fragment was then ligated to Not 1 cut phosphatased vector using T4 DNA ligase and the DNA transformed into E. coli TG-1. Clones containing inserts were identified as previously described.

3. DNA sequencing

The cDNA inserts from clones pBH5, pBH7, pBH8, pBH9, pBH10 and the PCR insert pBH6, were sequenced using the Sanger dideoxy chain terminations method as described by Barrell and Bankier (Methods in Enzymology 155, 51-93, 1987). This shotgun approach was supplemented as necessary with sequencing from specific oligonucleotide primers on double stranded or single stranded (f1 origin in pUC 119) plasmid DNA.

For shotgun analyses, insert DNA was excised from the vector sequences, selfligated, sonicated, end-repaired, size selected on 1% agarose gels, cloned into Sma 1-cut phosphatased M13mp18. Shotgun sequence data were assembled and analysed using the SAP programmes of Standen. A Vax 8350 and MicroVax 3100 (Digital Equipment Corporations) were used. The sequence data are presented SEQ ID NO.: 3.

Example 2

2.1. Generation of vaccinia virus Vac4b-C6

2.1.1. Assembly of CCV6 full length spike protein gene.

The full length coding region of the S gene of CCV6 was reconstructed from 2 overlapping cDNA clones, BH1 and BH2. The cloning strategy is illustrated in figure 1. The 3.0 kb insert from pBH1 has identity to S and 1b. In order to express S, the polymerase coding sequence had to be removed. The

sequence immediately 5' of the initiating methionine, CTAAACTTTGGTAATCACTTGG TTAATGTGCC ATG was modified by site directed mutagenesis. Four bases, ATCC were looped in between the TGG and TTA bases to create a unique BamHI site, GGATCC. Mutants were screened by restriction enzyme digestion. Positive clones were sequenced across this site as the Klenow fragment of E. coli DNA polymerase used in the mutagenesis reaction can introduce unspecified mutations at a very low frequency. A mutant which had the introduced BamHI site was selected and designated pBHI-bam. This plasmid overlapped pBH2 by approximately 300 bp. A unique AflII site was located in this region of overlap. The proximal S coding sequence was isolated from pBH1-bam as a 1.5 kb Af1II-SphI fragment and ligated into Af1II-SphI digested pBH2 generating pCCV6. The full length S coding sequence was isolated as a 4.4 kb BamH1 fragment then ligated into the BamH1 site of the transfer vector RK19 to form pRKCCV6. Correct orientation of the gene was confirmed by restriction enzyme digestion. Thus, the plasmid RKCCV6 contains the CCV6 S gene downstream of the 4b promoter and flanked by TK coding sequences.

2.1.2. Isolation of recombinant virus

Recombinant vaccinia viruses were constructed by established procedures (Mackett & Smith, J.Gen.Virol. 67, 2067-2082, 1986). pRKCCV6 was transfected into vaccinia virus infected cells and recombinant viruses identified by dot-blot hybridisation using random primed ^{32}P labelled CCV6 spike gene as a probe. Plaque purification and screening were repeated 3 times before stocks were prepared. The recombinant derived from pRKCCV6 was named Vac4b-C6.

2.2. Generation of vaccinia virus Vac4b-IN

2.2.1. Assembly of CCV Insavc-1 full length spike protein gene

The Insavc-1 (Bert) S gene was assembled from 3 overlapping cDNA clones BH8, BH9 and BH10. The cloning strategy is illustrated in figure 2. Digesting pBH8, which spans the middle of the S gene with PvuII and HindIII yielded a 1.4 kb fragment. This fragment was ligated into a PvuII-HindIII cut vector, pING14.2 forming pINGMS. This plasmid was linearized with HindIII, phosphatased then gel eluted. The 3' S gene coding sequence isolated as a 1.1 kb HindIII fragment from pBH10, was subcloned into HindIII cut pINGMS generating pINGM3'S. Correct orientation of the cloned HindIII fragment was confirmed by restriction enzyme digestion. Before the remaining coding sequence was excised from pBH9 a unique BamHI site was introduced 10bps upstream of the peplomer AUG start codon by site-directed mutagenesis (figure 2). The 5' coding sequence of the S gene was isolated as a 1.9 kb PvuII fragment and the remaining S gene coding sequence, which was isolated as a 2.5 kb PvuII partial-EcoRI fragment from pING3'S, were ligated in a two fragment ligation to BamHI-EcoRI digested pUC118. The complete S protein gene coding sequence was isolated as a 4.4 kb BamH1 fragment and subcloned into the BamH1 cut transfer vector pRK19, generating pRKINSAVC-1. Correct orientation of the gene was confirmed by restriction enzyme digestion. Thus the plasmid RKINSAVC-1 contains the CCV-INSAVC-1 S gene downstream of the vaccinia 4b promoter and flanked by TK coding sequences.

2.2.2. Isolation of recombinant vaccinia virus

Plasmid RKINSAVC-1 was used to introduce the S gene coding sequence into vaccinia virus by transfection and selection for TK^- recombinants was as described by Mackett and Smith, (1986, ibid). Recombinant virus isolates identified by dot blot hybridisation with a ^{32}P labelled CCV6 S DNA probe were subjected to three rounds of plaque purification and virus stocks prepared. The recombinant derived from RKINSAVC-1 was named Vac4b-IN.

2.3. Generation of vaccinia virus Vac4b-C54

2.3.1. Assembly of CCV C54 full length S protein gene

The C54 S gene coding sequence was assembled from the 3 overlapping clones pBH3, pBH4 and pBH11. A unique BamH1 site was created 10 bps upstream of the peplomer AUG start codon by site-directed mutagenesis in the proximal clone, pBH3 generating pBH3-bam (figure 3). A 2.0 kb AflII-EcoRI fragment was isolated from this plasmid and ligated to AflII-EcoRI digested pBH4 forming pBH5'MS. This plasmid was cleaved with HindIII, phosphatased and gel eluted. The 3' coding sequence was excised as a

1.1 kb HindIII fragment from pBH11, then ligated to the HindIII digested pBH5'MS generating pBHC54. The correct orientation of the subcloned HindIII fragment was determined by restriction enzyme digestion. The full length C54 S gene was excised by digestion with BamH1 from pBHC54 and ligated into the BamH1 cut transfer vector RK19, forming pRKC54. Similarly, the orientation of the S gene was determined by restriction enzyme digestion. Thus the plasmid RKC54 contains the CCV C54 S gene downstream of the 4b promoter and flanked by TK coding sequences. The cloning strategy is illustrated in figure 3.

2.3.2. Isolation of recombinant vaccinia virus

Plasmid RKC54 was transfected into vaccinia virus infected cells. TK^- recombinants were selected using BUdR (Mackett and Smith, 1986, ibid). Recombinant virus isolates were identified by dot-blot hybridisation and subjected to three rounds of plaque purification before stocks were made. The recombinant derived from pRKC54 was named Vac4b-C54.

Example 3

Immunization experiments with live recombinant Vaccinia vaccine

3.1. Immunization

Cats were vaccinated with the following vaccines (10^7 pfu/cat):

(a) 4 cats - Vac4b-IN
(b) 4 cats - Vac4b-C6
(c) 2 cats - Vac4b-gB
(Vac4b-gB is recombinant Vaccinia virus which expresses the Cytomegalovirus glycoprotein gB under control of the 4b promoter)
(d) 2 cats - unvaccinated.

All cats were bled prior to vaccination. (Bleed A). 3 weeks after vaccination the cats were bled again (Bleed B) and subsequently re-vaccinated as above.

2 weeks after re-vaccination all cats were bled (Bleed C).

3.2. Immuno-precipitation

Canine A72 cells were infected at a m.o.i. of about 10 with the recombinant viruses or mock-infected, incubated for 16 hours and starved of methionine for 1 hour. Infected cells were labelled with ^{35}S methionine and incubated for 30 min., washed and subsequently lysed in R.1.P.A. buffer. 1 μl cat antiserum (Bleed C) was added to the radiolabelled lysate and incubated on ice for 60 min. Protein G is added and incubated on ice for 60 min. After washing the protein G in R.1.P.A. buffer and PBS buffer, the bound proteins are recovered with 2% SDS 2% 2-mercapto-ethanol. The proteins are separated on 10% SDS polyacrylamide gel.

Sera from Bleed C precipitated the spike protein in the case of cats given Vac4b-C6 and Vac4b-IN. Thus, the cats immunized with the Vaccinia recombinant virus containing the spike genes responded with antibodies to the spike genes.

Legends to the Figures

Figure 1:	shows the cloning strategy for the construction of plasmid pRKCCV6 from plasmids pBH1 and pBH2.
Fugure 2:	shows the cloning strategy for the construction of plasmid pRKINSAVC-1 from plasmids pBH8, pBH10 and pBH9.
Figure 3:	shows the cloning strategy for the construction of plasmid pRKC54 from plasmids pBH3, pBH4 and pBH11.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: AKZO N.V.
(B) STREET: Velperweg 76
(C) CITY: Arnhem
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(F) POSTAL CODE (ZIP): 6824 BM

(ii) TITLE OF INVENTION: CANINE CORONAVIRUS SUBUNIT VACCINE

(iii) NUMBER OF SEQUENCES: 6

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: EP 91.303.737.0
(B) FILING DATE: 25-Apr-1991
(C) CLASSIFICATION:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4500 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Canine corona virus
(B) STRAIN: CCV-6

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 65..4393
(D) OTHER INFORMATION: /label= CCV6_Spikegene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GTAAGTTGCT CATTAGAAAC AATGGAAAAC TACTAAACTT TGGTAATCAC TTGGTTAATG      60

TGCC ATG ATT GTG CTA ATA TTG TGC CTC CTC TTG TTT TCG TAC AAT AGT     109
     Met Ile Val Leu Ile Leu Cys Leu Leu Leu Phe Ser Tyr Asn Ser
       1               5                  10                  15
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GTG ATT TGT ACA TCA AAC AAT GAC TGT GTA CAA GTT AAT GTG ACA CAA      157
Val Ile Cys Thr Ser Asn Asn Asp Cys Val Gln Val Asn Val Thr Gln
                 20                  25                  30

TTG CCT GGC AAT GAA AAC ATT ATT AAA GAT TTT CTA TTT CAC ACC TTC      205
Leu Pro Gly Asn Glu Asn Ile Ile Lys Asp Phe Leu Phe His Thr Phe
             35                  40                  45

AAA GAA GAA GGA AGT GTA GTT GTT GGT GGT TAT TAC CCT ACA GAG GTG      253
Lys Glu Glu Gly Ser Val Val Val Gly Gly Tyr Tyr Pro Thr Glu Val
         50                  55                  60

TGG TAT AAC TGC TCC AGA AGC GCA ACA ACC ACC GCT TAC AAG GAT TTT      301
Trp Tyr Asn Cys Ser Arg Ser Ala Thr Thr Thr Ala Tyr Lys Asp Phe
     65                  70                  75

AGT AAT ATA CAT GCA TTC TAT TTT GAT ATG GAA GAC ATG GAG AAA AGC      349
Ser Asn Ile His Ala Phe Tyr Phe Asp Met Glu Asp Met Glu Lys Ser
 80                  85                  90                  95

ACT GGC AAT GCA CGA GGA AAA CCT TTA CTA GTA CAT GTT CAT GGT GGA      397
Thr Gly Asn Ala Arg Gly Lys Pro Leu Leu Val His Val His Gly Gly
                100                 105                 110

CCT GTT AGT ATC ATC ATT ATA TGT GCA AGG AAG GCC TCT TTA AAA CAT      445
Pro Val Ser Ile Ile Ile Ile Cys Ala Arg Lys Ala Ser Leu Lys His
            115                 120                 125

GGT TTG TTG TGT ATA ACT AAA AAT AAA ATC ATT GAC TAT AAC ACG TTT      493
Gly Leu Leu Cys Ile Thr Lys Asn Lys Ile Ile Asp Tyr Asn Thr Phe
        130                 135                 140

ACC AGC GCA CAG TGG AGT GCC ATA TGT TTG GGT GAT GAC AGA AAA ATA      541
Thr Ser Ala Gln Trp Ser Ala Ile Cys Leu Gly Asp Asp Arg Lys Ile
    145                 150                 155

CCA TTC TCT GTC ATA CCC ACA GAT AAT GGT ACA AAA ATA TTT GGT CTT      589
Pro Phe Ser Val Ile Pro Thr Asp Asn Gly Thr Lys Ile Phe Gly Leu
160                 165                 170                 175

GAG TGG AAT GAT GAC TAT GTT ACA GCC TAT ATT AGT GAT CGT TCT CAC      637
Glu Trp Asn Asp Asp Tyr Val Thr Ala Tyr Ile Ser Asp Arg Ser His
                180                 185                 190

CAT TTG AAC ATC AAT AAT AAT TGG TTT AAC AAT GTG ACA ATC CTA TAC      685
His Leu Asn Ile Asn Asn Asn Trp Phe Asn Asn Val Thr Ile Leu Tyr
            195                 200                 205

TCT CGA TCA AGC ACT GCT ACG TGG CAG AAG AGT GCT GCA TAT GTT TAT      733
Ser Arg Ser Ser Thr Ala Thr Trp Gln Lys Ser Ala Ala Tyr Val Tyr
        210                 215                 220

CAA GGT GTT TCA AAT TTT ACT TAT TAC AAG TTA AAT AAC ACC AAT GGC      781
Gln Gly Val Ser Asn Phe Thr Tyr Tyr Lys Leu Asn Asn Thr Asn Gly
    225                 230                 235
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TTG AAA AGC TAT GAA TTG TGT GAA GAT TAT GAA TAC TGC ACT GGC TAT     829
Leu Lys Ser Tyr Glu Leu Cys Glu Asp Tyr Glu Tyr Cys Thr Gly Tyr
240                 245                 250                 255

GCT ACC AAC GTA TTT GCC CCG ACA GTG GGC GGT TAT ATA CCT GAT GGC     877
Ala Thr Asn Val Phe Ala Pro Thr Val Gly Gly Tyr Ile Pro Asp Gly
                260                 265                 270

TTC AGT TTT AAC AAT TGG TTT ATG CTT ACA AAC AGT TCC ACG TTT GTT     925
Phe Ser Phe Asn Asn Trp Phe Met Leu Thr Asn Ser Ser Thr Phe Val
            275                 280                 285

AGT GGC AGA TTT GTA ACA AAT CAA CCA TTA TTG GTT AAT TGT TTG TGG     973
Ser Gly Arg Phe Val Thr Asn Gln Pro Leu Leu Val Asn Cys Leu Trp
        290                 295                 300

CCA GTG CCC AGT TTT GGT GTC GCA GCA CAA GAA TTT TGT TTT GAA GGT    1021
Pro Val Pro Ser Phe Gly Val Ala Ala Gln Glu Phe Cys Phe Glu Gly
    305                 310                 315

GCG CAG TTT AGC CAA TGT AAT GGT GTG TCT TTA AAC AAT ACA GTG GAT    1069
Ala Gln Phe Ser Gln Cys Asn Gly Val Ser Leu Asn Asn Thr Val Asp
320                 325                 330                 335

GTC ATT AGA TTC AAC CTT AAT TTT ACC ACA GAT GTA CAA TCT GGC ATG    1117
Val Ile Arg Phe Asn Leu Asn Phe Thr Thr Asp Val Gln Ser Gly Met
                340                 345                 350

GGT GCT ATA GTA TTT TCA CTG AAT ACA ACA GGT GGT GTC ATT CTT GAG    1165
Gly Ala Ile Val Phe Ser Leu Asn Thr Thr Gly Gly Val Ile Leu Glu
            355                 360                 365

ATT TCT TGT TAT AAT GAT ACA GTG AGT GAG TCA AGT TTC TAC AGT TAT    1213
Ile Ser Cys Tyr Asn Asp Thr Val Ser Glu Ser Ser Phe Tyr Ser Tyr
        370                 375                 380

GGT GAA ATT TCA ATC GGC GTA ACT GAT GGA CCG CGT TAC TGT TAC GCC    1261
Gly Glu Ile Ser Ile Gly Val Thr Asp Gly Pro Arg Tyr Cys Tyr Ala
    385                 390                 395

CTC TAT AAT GGC CAG GCT CTT AAG TGT TTA GGA ACA TTA CCA CCT AGT    1309
Leu Tyr Asn Gly Gln Ala Leu Lys Cys Leu Gly Thr Leu Pro Pro Ser
400                 405                 410                 415

GTC AAG GAA ATT GCT ATT AGT AAG TGG GGC CAT TTT TAT ATT AAT GGT    1357
Val Lys Glu Ile Ala Ile Ser Lys Trp Gly His Phe Tyr Ile Asn Gly
                420                 425                 430

TAC AAT TTC TTT AGC ACT TTT CCT ATT GAT TGT ATA TCT TTT AAT TTA    1405
Tyr Asn Phe Phe Ser Thr Phe Pro Ile Asp Cys Ile Ser Phe Asn Leu
            435                 440                 445

ACC ACT GGT GAT AGT GGA GCA TTT TGG ACA ATT GCT TAC ACA TCG TAC    1453
Thr Thr Gly Asp Ser Gly Ala Phe Trp Thr Ile Ala Tyr Thr Ser Tyr
        450                 455                 460
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ACT GAC GCA TTA GTA CAA GTT GAA AAC ACA GCT ATT AAA AAG GTG ACG      1501
Thr Asp Ala Leu Val Gln Val Glu Asn Thr Ala Ile Lys Lys Val Thr
    465                 470                 475

TAT TGT AAC AGT CAC ATT AAT AAC ATT AAA TGT TCT CAA CTT ACT GCT      1549
Tyr Cys Asn Ser His Ile Asn Asn Ile Lys Cys Ser Gln Leu Thr Ala
480                 485                 490                 495

AAT TTG CAA AAT GGA TTT TAT CCT GTT GCT TCA AGT GAA GTT GGT CTT      1597
Asn Leu Gln Asn Gly Phe Tyr Pro Val Ala Ser Ser Glu Val Gly Leu
                500                 505                 510

GTC AAT AAG AGT GTT GTG TTA CTA CCT AGT TTC TAT TCA CAT ACC AGT      1645
Val Asn Lys Ser Val Val Leu Leu Pro Ser Phe Tyr Ser His Thr Ser
            515                 520                 525

GTT AAT ATA ACT ATT GAT CTT GGT ATG AAG CGT AGT GTT ATG GTC ACC      1693
Val Asn Ile Thr Ile Asp Leu Gly Met Lys Arg Ser Val Met Val Thr
        530                 535                 540

ATA GCC TCA ACA TTA AGT AAC ATC ACA CTA CCA ATG CAG GAT AAT AAC      1741
Ile Ala Ser Thr Leu Ser Asn Ile Thr Leu Pro Met Gln Asp Asn Asn
    545                 550                 555

ACC GAT GTG TAC TGC ATT CGT TCT AAC CAA TTT TCA GTT TAC GTT CAT      1789
Thr Asp Val Tyr Cys Ile Arg Ser Asn Gln Phe Ser Val Tyr Val His
560                 565                 570                 575

TCC ACT TGT AAA AGT TCT TTA TGG GAC GAT GTG TTT AAT TCC GAC TGC      1837
Ser Thr Cys Lys Ser Ser Leu Trp Asp Asp Val Phe Asn Ser Asp Cys
                580                 585                 590

ACA GAT GTT TTA TAT GCT ACA GCT GTT ATA AAA ACT GGT ACT TGT CCT      1885
Thr Asp Val Leu Tyr Ala Thr Ala Val Ile Lys Thr Gly Thr Cys Pro
            595                 600                 605

TTC TCG TTT GAT AAA TTG AAC AAT TAC TTA ACT TTT AAC AAG TTC TGT      1933
Phe Ser Phe Asp Lys Leu Asn Asn Tyr Leu Thr Phe Asn Lys Phe Cys
        610                 615                 620

TTG TCA TTG AAT CCT GTT GGT GCC AAC TGC AAG TTT GAT GTT GCC GCT      1981
Leu Ser Leu Asn Pro Val Gly Ala Asn Cys Lys Phe Asp Val Ala Ala
    625                 630                 635

CGT ACA AGA ACC AAT GAG CAG GTT GTT AGA AGT TTA TAT GTA ATA TAT      2029
Arg Thr Arg Thr Asn Glu Gln Val Val Arg Ser Leu Tyr Val Ile Tyr
640                 645                 650                 655

GAA GAA GGA GAC AAC ATA GCG GGT GTG CCG TCT GAC AAT AGT GGT CTT      2077
Glu Glu Gly Asp Asn Ile Ala Gly Val Pro Ser Asp Asn Ser Gly Leu
                660                 665                 670

CAC GAC TTG TCA GTG CTA CAC TTA GAC TCC TGT ACA GAT TAT AAT ATA      2125
His Asp Leu Ser Val Leu His Leu Asp Ser Cys Thr Asp Tyr Asn Ile
            675                 680                 685
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TAT GGT AGA ACT GGT GTT GGT ATT ATT AGA CAA ACT AAC AGT ACG CTA    2173
Tyr Gly Arg Thr Gly Val Gly Ile Ile Arg Gln Thr Asn Ser Thr Leu
        690                 695                 700

CTT AGT GGC TTA TAT TAC ACA TCA CTA TCA GGT GAC TTG TTA GGG TTT    2221
Leu Ser Gly Leu Tyr Tyr Thr Ser Leu Ser Gly Asp Leu Leu Gly Phe
    705                 710                 715

AAA AAT GTT AGT GAT GGT GTC ATC TAT TCT GTC ACG CCA TGT GAT GTA    2269
Lys Asn Val Ser Asp Gly Val Ile Tyr Ser Val Thr Pro Cys Asp Val
720                 725                 730                 735

AGC GTA CAA GCT GCT GTT ATT GAT GGC GCC ATA GTT GGA GCT ATG ACT    2317
Ser Val Gln Ala Ala Val Ile Asp Gly Ala Ile Val Gly Ala Met Thr
                740                 745                 750

TCC ATT AAT AGT GAA CTG TTA GGT CTA ACA CAT TGG ACA ACA ACA CCT    2365
Ser Ile Asn Ser Glu Leu Leu Gly Leu Thr His Trp Thr Thr Thr Pro
            755                 760                 765

AAT TTT TAT TAT TAT TCT ATA TAT AAT TAT ACC AAT GAA AGG ACT CGT    2413
Asn Phe Tyr Tyr Tyr Ser Ile Tyr Asn Tyr Thr Asn Glu Arg Thr Arg
        770                 775                 780

GGC ACA GCA ATT GAT AGT AAC GAT GTT GAT TGT GAA CCT ATC ATA ACC    2461
Gly Thr Ala Ile Asp Ser Asn Asp Val Asp Cys Glu Pro Ile Ile Thr
    785                 790                 795

TAT TCT AAT ATA GGT GTT TGT AAA AAT GGA GCT TTG GTT TTT ATT AAC    2509
Tyr Ser Asn Ile Gly Val Cys Lys Asn Gly Ala Leu Val Phe Ile Asn
800                 805                 810                 815

GTC ACA CAT TCT GAT GGA GAC GTT CAA CCA ATT AGC ACC GGT AAT GTC    2557
Val Thr His Ser Asp Gly Asp Val Gln Pro Ile Ser Thr Gly Asn Val
                820                 825                 830

ACG ATA CCT ACA AAT TTT ACC ATA TCT GTG CAA GTT GAA TAC ATT CAG    2605
Thr Ile Pro Thr Asn Phe Thr Ile Ser Val Gln Val Glu Tyr Ile Gln
            835                 840                 845

GTT TAC ACT ACA CCG GTG TCA ATA GAT TGT TCA AGG TAC GTT TGC AAT    2653
Val Tyr Thr Thr Pro Val Ser Ile Asp Cys Ser Arg Tyr Val Cys Asn
        850                 855                 860

GGT AAC CCT AGA TGC AAT AAA TTG TTA ACG CAA TAC GTT TCT GCA TGT    2701
Gly Asn Pro Arg Cys Asn Lys Leu Leu Thr Gln Tyr Val Ser Ala Cys
    865                 870                 875

CAA ACT ATT GAG CAA GCA CTT GCA ATG GGT GCC AGA CTT GAA AAC ATG    2749
Gln Thr Ile Glu Gln Ala Leu Ala Met Gly Ala Arg Leu Glu Asn Met
880                 885                 890                 895

GAG ATT GAT TCC ATG TTG TTT GTT TCG GAA AAT GCC CTT AAA TTG GCA    2797
Glu Ile Asp Ser Met Leu Phe Val Ser Glu Asn Ala Leu Lys Leu Ala
                900                 905                 910
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TCT GTT GAA GCA TTA ATA GTA GGA AAT TTA GAT CCT ATT TAC AAA GAA      2845
Ser Val Glu Ala Leu Ile Val Gly Asn Leu Asp Pro Ile Tyr Lys Glu
            915                 920                 925

TGG CCT AAC ATT GGT GGT TCT TGG CTA GGA GGT TTA AAA GAC ATA TTG      2893
Trp Pro Asn Ile Gly Gly Ser Trp Leu Gly Gly Leu Lys Asp Ile Leu
        930                 935                 940

CCA TCT CAC AAC AGC AAA CGT AAG TAC CGG TCG GCT ATA GAA GAT TTG      2941
Pro Ser His Asn Ser Lys Arg Lys Tyr Arg Ser Ala Ile Glu Asp Leu
    945                 950                 955

CTT TTT GAT AAG GTT GTA ACA TCT GGC TTA GGT ACA GTT GAT GAA GAT      2989
Leu Phe Asp Lys Val Val Thr Ser Gly Leu Gly Thr Val Asp Glu Asp
960                 965                 970                 975

TAT AAA CGT TGT ACA GGT GGT TAT GAC ATA GCT GAC TTA GTG TGT GCA      3037
Tyr Lys Arg Cys Thr Gly Gly Tyr Asp Ile Ala Asp Leu Val Cys Ala
                980                 985                 990

CAA TAT TAC AAT GGC ATC ATG GTG CTA CCT GGT GTA GCT AAT GAT GAC      3085
Gln Tyr Tyr Asn Gly Ile Met Val Leu Pro Gly Val Ala Asn Asp Asp
            995                1000                1005

AAG ATG GCT ATG TAC ACT GCA TCT CTT GCA GGT GGT ATA ACA TTA GGT      3133
Lys Met Ala Met Tyr Thr Ala Ser Leu Ala Gly Gly Ile Thr Leu Gly
        1010                1015                1020

GCA CTT GGT GGT GGC GCA GTG TCT ATA CCT TTT GCA ATA GCA GTT CAA      3181
Ala Leu Gly Gly Gly Ala Val Ser Ile Pro Phe Ala Ile Ala Val Gln
    1025                1030                1035

GCC AGA CTT AAT TAT GTT GCT CTA CAA ACT GAT GTA TTG AAC AAG AAC      3229
Ala Arg Leu Asn Tyr Val Ala Leu Gln Thr Asp Val Leu Asn Lys Asn
1040                1045                1050                1055

CAG CAG ATC CTG GCT AAT GCT TTC AAT CAA GCT ATT GGT AAC ATT ACA      3277
Gln Gln Ile Leu Ala Asn Ala Phe Asn Gln Ala Ile Gly Asn Ile Thr
                1060                1065                1070

CAG GCA TTT GGT AAG GTT AAT GAT GCT ATA CAT CAA ACG TCA CAA GGT      3325
Gln Ala Phe Gly Lys Val Asn Asp Ala Ile His Gln Thr Ser Gln Gly
            1075                1080                1085

CTT GCT ACT GTT GCT AAA GCA TTG GCA AAA GTG CAA GAT GTT GTT AAC      3373
Leu Ala Thr Val Ala Lys Ala Leu Ala Lys Val Gln Asp Val Val Asn
        1090                1095                1100

ACA CAA GGG CAA GCT TTA AGC CAC CTA ACA GTA CAA TTG CAA AAT AAT      3421
Thr Gln Gly Gln Ala Leu Ser His Leu Thr Val Gln Leu Gln Asn Asn
    1105                1110                1115

TTC CAA GCC ATT AGT AGT TCC ATT AGT GAC ATT TAT AAC AGG CTT GAT      3469
Phe Gln Ala Ile Ser Ser Ser Ile Ser Asp Ile Tyr Asn Arg Leu Asp
1120                1125                1130                1135
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GAA TTG AGT GCT GAT GCA CAA GTT GAC AGG CTG ATT ACA GGA AGA CTT      3517
Glu Leu Ser Ala Asp Ala Gln Val Asp Arg Leu Ile Thr Gly Arg Leu
                1140                1145                1150

ACA GCA CTT AAT GCA TTT GTG TCT CAG ACT TTA ACC AGA CAA GCA GAG      3565
Thr Ala Leu Asn Ala Phe Val Ser Gln Thr Leu Thr Arg Gln Ala Glu
            1155                1160                1165

GTT AGG GCT AGC AGA CAG CTT GCT AAA GAC AAG GTA AAT GAA TGC GTT      3613
Val Arg Ala Ser Arg Gln Leu Ala Lys Asp Lys Val Asn Glu Cys Val
        1170                1175                1180

AGG TCT CAA TCT CAG AGA TTT GGA TTC TGT GGT AAT GGT ACA CAT TTA      3661
Arg Ser Gln Ser Gln Arg Phe Gly Phe Cys Gly Asn Gly Thr His Leu
    1185                1190                1195

TTT TCA CTT GCA AAT GCA GCA CCA AAT GGC ATG ATC TTC TTT CAC ACA      3709
Phe Ser Leu Ala Asn Ala Ala Pro Asn Gly Met Ile Phe Phe His Thr
1200                1205                1210                1215

GTG CTA TTA CCA ACA GCT TAT GAA ACC GTG ACA GCC TGG TCA GGT ATT      3757
Val Leu Leu Pro Thr Ala Tyr Glu Thr Val Thr Ala Trp Ser Gly Ile
                1220                1225                1230

TGT GCA TCA GAT GGC GAT CGT ACT TTT GGA CTT GTT GTT AAG GAT GTC      3805
Cys Ala Ser Asp Gly Asp Arg Thr Phe Gly Leu Val Val Lys Asp Val
            1235                1240                1245

CAG TTG ACG CTG TTT CGC AAT CTA GAT GAC AAA TTC TAT TTG ACT CCC      3853
Gln Leu Thr Leu Phe Arg Asn Leu Asp Asp Lys Phe Tyr Leu Thr Pro
        1250                1255                1260

AGA ACT ATG TAT CAG CCT AGA GTT GCA ACT AGT TCT GAT TTT GTT CAA      3901
Arg Thr Met Tyr Gln Pro Arg Val Ala Thr Ser Ser Asp Phe Val Gln
    1265                1270                1275

ATT GAA GGA TGT GAT GTG TTG TTT GTT AAT GCA ACT GTA ATT GAC TTG      3949
Ile Glu Gly Cys Asp Val Leu Phe Val Asn Ala Thr Val Ile Asp Leu
1280                1285                1290                1295

CCT AGT ATT ATA CCT GAC TAT ATT GAT ATT AAT CAA ACT GTT CAG GAC      3997
Pro Ser Ile Ile Pro Asp Tyr Ile Asp Ile Asn Gln Thr Val Gln Asp
                1300                1305                1310

ATA TTA GAA AAT TTC AGA CCA AAT TGG ACT GTA CCT GAG TTG CCA CTT      4045
Ile Leu Glu Asn Phe Arg Pro Asn Trp Thr Val Pro Glu Leu Pro Leu
            1315                1320                1325

GAC ATT TTC AAT GCA ACC TAC TTA AAC CTG ACT GGT GAA ATT AAG TGC      4093
Asp Ile Phe Asn Ala Thr Tyr Leu Asn Leu Thr Gly Glu Ile Lys Cys
        1330                1335                1340

TTA GAA TTT AGG TCA GAA AAG TTA CAT AAC ACC ACA GTA GAA CTT GCT      4141
Leu Glu Phe Arg Ser Glu Lys Leu His Asn Thr Thr Val Glu Leu Ala
    1345                1350                1355
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ATT CTC ATT GAT AAT ATT AAT AAC ACA TTA TCA ATC TTA ATG CTC AAT      4189
Ile Leu Ile Asp Asn Ile Asn Asn Thr Leu Ser Ile Leu Met Leu Asn
1360                1365                1370                1375

AGA ATT GAA ACT TAT GTA AAA TGG CCT TGG TAT GTG TGG CTA CTA ATT      4237
Arg Ile Glu Thr Tyr Val Lys Trp Pro Trp Tyr Val Trp Leu Leu Ile
                1380                1385                1390

GGA TTA GTA GTA ATA TTC TGC ATA CCC ATA TTG CTA TTT TGT TGT TGT      4285
Gly Leu Val Val Ile Phe Cys Ile Pro Ile Leu Leu Phe Cys Cys Cys
            1395                1400                1405

AGT ACT GGT TGT TGT GGA TGT ATT GGG TGT TTA GGA AGC TGT TGT CAT      4333
Ser Thr Gly Cys Cys Gly Cys Ile Gly Cys Leu Gly Ser Cys Cys His
        1410                1415                1420

TCC ATA TGT AGT AGA AGG CAA TTT GAA AGT TAT GAA CCA ATT GAA AAA      4381
Ser Ile Cys Ser Arg Arg Gln Phe Glu Ser Tyr Glu Pro Ile Glu Lys
    1425                1430                1435

GTT CAT GTT CAC TGAATTCAAA ATGTTAAGTC TACTATTTTA ATTACACCCG          4433
Val His Val His
1440

TGGCCACACA AGTTATATAA TGGTGCTGTC GTAAGTTCGA TACCAGTCAA CTATTAGCAT    4493

TAATAAA                                                              4500
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1443 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Canina corona virus
(B) STRAIN: CCV-6

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..1443
(D) OTHER INFORMATION: /label= CCV6_Spike

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ile Val Leu Ile Leu Cys Leu Leu Leu Phe Ser Tyr Asn Ser Val
1               5                   10                  15
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Ile Cys Thr Ser Asn Asn Asp Cys Val Gln Val Asn Val Thr Gln Leu
            20                  25                  30

Pro Gly Asn Glu Asn Ile Ile Lys Asp Phe Leu Phe His Thr Phe Lys
        35                  40                  45

Glu Glu Gly Ser Val Val Val Gly Gly Tyr Tyr Pro Thr Glu Val Trp
    50                  55                  60

Tyr Asn Cys Ser Arg Ser Ala Thr Thr Thr Ala Tyr Lys Asp Phe Ser
65                  70                  75                  80

Asn Ile His Ala Phe Tyr Phe Asp Met Glu Asp Met Glu Lys Ser Thr
                85                  90                  95

Gly Asn Ala Arg Gly Lys Pro Leu Leu Val His Val His Gly Gly Pro
            100                 105                 110

Val Ser Ile Ile Ile Ile Cys Ala Arg Lys Ala Ser Leu Lys His Gly
        115                 120                 125

Leu Leu Cys Ile Thr Lys Asn Lys Ile Ile Asp Tyr Asn Thr Phe Thr
    130                 135                 140

Ser Ala Gln Trp Ser Ala Ile Cys Leu Gly Asp Asp Arg Lys Ile Pro
145                 150                 155                 160

Phe Ser Val Ile Pro Thr Asp Asn Gly Thr Lys Ile Phe Gly Leu Glu
                165                 170                 175

Trp Asn Asp Asp Tyr Val Thr Ala Tyr Ile Ser Asp Arg Ser His His
            180                 185                 190

Leu Asn Ile Asn Asn Asn Trp Phe Asn Asn Val Thr Ile Leu Tyr Ser
        195                 200                 205

Arg Ser Ser Thr Ala Thr Trp Gln Lys Ser Ala Ala Tyr Val Tyr Gln
    210                 215                 220

Gly Val Ser Asn Phe Thr Tyr Tyr Lys Leu Asn Asn Thr Asn Gly Leu
225                 230                 235                 240

Lys Ser Tyr Glu Leu Cys Glu Asp Tyr Glu Tyr Cys Thr Gly Tyr Ala
                245                 250                 255

Thr Asn Val Phe Ala Pro Thr Val Gly Gly Tyr Ile Pro Asp Gly Phe
            260                 265                 270

Ser Phe Asn Asn Trp Phe Met Leu Thr Asn Ser Ser Thr Phe Val Ser
        275                 280                 285

Gly Arg Phe Val Thr Asn Gln Pro Leu Leu Val Asn Cys Leu Trp Pro
    290                 295                 300

Val Pro Ser Phe Gly Val Ala Ala Gln Glu Phe Cys Phe Glu Gly Ala
305                 310                 315                 320
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Gln Phe Ser Gln Cys Asn Gly Val Ser Leu Asn Asn Thr Val Asp Val
325 330 335

Ile Arg Phe Asn Leu Asn Phe Thr Thr Asp Val Gln Ser Gly Met Gly
340 345 350

Ala Ile Val Phe Ser Leu Asn Thr Thr Gly Gly Val Ile Leu Glu Ile
355 360 365

Ser Cys Tyr Asn Asp Thr Val Ser Glu Ser Ser Phe Tyr Ser Tyr Gly
370 375 380

Glu Ile Ser Ile Gly Val Thr Asp Gly Pro Arg Tyr Cys Tyr Ala Leu
385 390 395 400

Tyr Asn Gly Gln Ala Leu Lys Cys Leu Gly Thr Leu Pro Pro Ser Val
405 410 415

Lys Glu Ile Ala Ile Ser Lys Trp Gly His Phe Tyr Ile Asn Gly Tyr
420 425 430

Asn Phe Phe Ser Thr Phe Pro Ile Asp Cys Ile Ser Phe Asn Leu Thr
435 440 445

Thr Gly Asp Ser Gly Ala Phe Trp Thr Ile Ala Tyr Thr Ser Tyr Thr
450 455 460

Asp Ala Leu Val Gln Val Glu Asn Thr Ala Ile Lys Lys Val Thr Tyr
465 470 475 480

Cys Asn Ser His Ile Asn Asn Ile Lys Cys Ser Gln Leu Thr Ala Asn
485 490 495

Leu Gln Asn Gly Phe Tyr Pro Val Ala Ser Ser Glu Val Gly Leu Val
500 505 510

Asn Lys Ser Val Val Leu Leu Pro Ser Phe Tyr Ser His Thr Ser Val
515 520 525

Asn Ile Thr Ile Asp Leu Gly Met Lys Arg Ser Val Met Val Thr Ile
530 535 540

Ala Ser Thr Leu Ser Asn Ile Thr Leu Pro Met Gln Asp Asn Asn Thr
545 550 555 560

Asp Val Tyr Cys Ile Arg Ser Asn Gln Phe Ser Val Tyr Val His Ser
565 570 575

Thr Cys Lys Ser Ser Leu Trp Asp Asp Val Phe Asn Ser Asp Cys Thr
580 585 590

Asp Val Leu Tyr Ala Thr Ala Val Ile Lys Thr Gly Thr Cys Pro Phe
595 600 605

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Ser Phe Asp Lys Leu Asn Asn Tyr Leu Thr Phe Asn Lys Phe Cys Leu
    610                 615                 620

Ser Leu Asn Pro Val Gly Ala Asn Cys Lys Phe Asp Val Ala Ala Arg
625                 630                 635                 640

Thr Arg Thr Asn Glu Gln Val Val Arg Ser Leu Tyr Val Ile Tyr Glu
                645                 650                 655

Glu Gly Asp Asn Ile Ala Gly Val Pro Ser Asp Asn Ser Gly Leu His
            660                 665                 670

Asp Leu Ser Val Leu His Leu Asp Ser Cys Thr Asp Tyr Asn Ile Tyr
        675                 680                 685

Gly Arg Thr Gly Val Gly Ile Ile Arg Gln Thr Asn Ser Thr Leu Leu
    690                 695                 700

Ser Gly Leu Tyr Tyr Thr Ser Leu Ser Gly Asp Leu Leu Gly Phe Lys
705                 710                 715                 720

Asn Val Ser Asp Gly Val Ile Tyr Ser Val Thr Pro Cys Asp Val Ser
                725                 730                 735

Val Gln Ala Ala Val Ile Asp Gly Ala Ile Val Gly Ala Met Thr Ser
            740                 745                 750

Ile Asn Ser Glu Leu Leu Gly Leu Thr His Trp Thr Thr Thr Pro Asn
        755                 760                 765

Phe Tyr Tyr Tyr Ser Ile Tyr Asn Tyr Thr Asn Glu Arg Thr Arg Gly
    770                 775                 780

Thr Ala Ile Asp Ser Asn Asp Val Asp Cys Glu Pro Ile Ile Thr Tyr
785                 790                 795                 800

Ser Asn Ile Gly Val Cys Lys Asn Gly Ala Leu Val Phe Ile Asn Val
                805                 810                 815

Thr His Ser Asp Gly Asp Val Gln Pro Ile Ser Thr Gly Asn Val Thr
            820                 825                 830

Ile Pro Thr Asn Phe Thr Ile Ser Val Gln Val Glu Tyr Ile Gln Val
        835                 840                 845

Tyr Thr Thr Pro Val Ser Ile Asp Cys Ser Arg Tyr Val Cys Asn Gly
    850                 855                 860

Asn Pro Arg Cys Asn Lys Leu Leu Thr Gln Tyr Val Ser Ala Cys Gln
865                 870                 875                 880

Thr Ile Glu Gln Ala Leu Ala Met Gly Ala Arg Leu Glu Asn Met Glu
                885                 890                 895

Ile Asp Ser Met Leu Phe Val Ser Glu Asn Ala Leu Lys Leu Ala Ser
            900                 905                 910
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Val Glu Ala Leu Ile Val Gly Asn Leu Asp Pro Ile Tyr Lys Glu Trp
        915                 920                 925

Pro Asn Ile Gly Gly Ser Trp Leu Gly Gly Leu Lys Asp Ile Leu Pro
    930                 935                 940

Ser His Asn Ser Lys Arg Lys Tyr Arg Ser Ala Ile Glu Asp Leu Leu
945                 950                 955                 960

Phe Asp Lys Val Val Thr Ser Gly Leu Gly Thr Val Asp Glu Asp Tyr
                965                 970                 975

Lys Arg Cys Thr Gly Gly Tyr Asp Ile Ala Asp Leu Val Cys Ala Gln
            980                 985                 990

Tyr Tyr Asn Gly Ile Met Val Leu Pro Gly Val Ala Asn Asp Asp Lys
        995                 1000                1005

Met Ala Met Tyr Thr Ala Ser Leu Ala Gly Gly Ile Thr Leu Gly Ala
    1010                1015                1020

Leu Gly Gly Gly Ala Val Ser Ile Pro Phe Ala Ile Ala Val Gln Ala
1025                1030                1035                1040

Arg Leu Asn Tyr Val Ala Leu Gln Thr Asp Val Leu Asn Lys Asn Gln
                1045                1050                1055

Gln Ile Leu Ala Asn Ala Phe Asn Gln Ala Ile Gly Asn Ile Thr Gln
            1060                1065                1070

Ala Phe Gly Lys Val Asn Asp Ala Ile His Gln Thr Ser Gln Gly Leu
        1075                1080                1085

Ala Thr Val Ala Lys Ala Leu Ala Lys Val Gln Asp Val Val Asn Thr
    1090                1095                1100

Gln Gly Gln Ala Leu Ser His Leu Thr Val Gln Leu Gln Asn Asn Phe
1105                1110                1115                1120

Gln Ala Ile Ser Ser Ser Ile Ser Asp Ile Tyr Asn Arg Leu Asp Glu
                1125                1130                1135

Leu Ser Ala Asp Ala Gln Val Asp Arg Leu Ile Thr Gly Arg Leu Thr
            1140                1145                1150

Ala Leu Asn Ala Phe Val Ser Gln Thr Leu Thr Arg Gln Ala Glu Val
        1155                1160                1165

Arg Ala Ser Arg Gln Leu Ala Lys Asp Lys Val Asn Glu Cys Val Arg
    1170                1175                1180

Ser Gln Ser Gln Arg Phe Gly Phe Cys Gly Asn Gly Thr His Leu Phe
1185                1190                1195                1200
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Ser Leu Ala Asn Ala Ala Pro Asn Gly Met Ile Phe Phe His Thr Val
1205 1210 1215

Leu Leu Pro Thr Ala Tyr Glu Thr Val Thr Ala Trp Ser Gly Ile Cys
1220 1225 1230

Ala Ser Asp Gly Asp Arg Thr Phe Gly Leu Val Val Lys Asp Val Gln
1235 1240 1245

Leu Thr Leu Phe Arg Asn Leu Asp Asp Lys Phe Tyr Leu Thr Pro Arg
1250 1255 1260

Thr Met Tyr Gln Pro Arg Val Ala Thr Ser Ser Asp Phe Val Gln Ile
1265 1270 1275 1280

Glu Gly Cys Asp Val Leu Phe Val Asn Ala Thr Val Ile Asp Leu Pro
1285 1290 1295

Ser Ile Ile Pro Asp Tyr Ile Asp Ile Asn Gln Thr Val Gln Asp Ile
1300 1305 1310

Leu Glu Asn Phe Arg Pro Asn Trp Thr Val Pro Glu Leu Pro Leu Asp
1315 1320 1325

Ile Phe Asn Ala Thr Tyr Leu Asn Leu Thr Gly Glu Ile Lys Cys Leu
1330 1335 1340

Glu Phe Arg Ser Glu Lys Leu His Asn Thr Thr Val Glu Leu Ala Ile
1345 1350 1355 1360

Leu Ile Asp Asn Ile Asn Asn Thr Leu Ser Ile Leu Met Leu Asn Arg
1365 1370 1375

Ile Glu Thr Tyr Val Lys Trp Pro Trp Tyr Val Trp Leu Leu Ile Gly
1380 1385 1390

Leu Val Val Ile Phe Cys Ile Pro Ile Leu Leu Phe Cys Cys Cys Ser
1395 1400 1405

Thr Gly Cys Cys Gly Cys Ile Gly Cys Leu Gly Ser Cys Cys His Ser
1410 1415 1420

Ile Cys Ser Arg Arg Gln Phe Glu Ser Tyr Glu Pro Ile Glu Lys Val
1425 1430 1435 1440

His Val His

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4429 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Canina corona virus
(B) STRAIN: CCVInSAVC-1

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 60..4412
(D) OTHER INFORMATION: /label= CCVInSAVC-1_Spikegene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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TTGCTCATTA GAAACAATGG TAAACTACTA AACTTTGGTA ATCACTTGGT TAATGTGCC       59

ATG ATT GTG CTT ACA TTG TGC CTT TTC TTG TTT TTG TAC AGT AGT GTG       107
Met Ile Val Leu Thr Leu Cys Leu Phe Leu Phe Leu Tyr Ser Ser Val
  1               5                  10                  15

AGC TGT ACA TCA AAC AAT GAC TGT GTA CAA GTT AAT GTG ACA CAA CTG       155
Ser Cys Thr Ser Asn Asn Asp Cys Val Gln Val Asn Val Thr Gln Leu
             20                  25                  30

CCT GGC AAT GAA AAT ATT ATC AAA GAT TTT CTA TTT CAG AAC TTT AAA       203
Pro Gly Asn Glu Asn Ile Ile Lys Asp Phe Leu Phe Gln Asn Phe Lys
         35                  40                  45

GAA GAA GGA AGT TTA GTT GTT GGT GGT TAT TAC CCC ACA GAG GTG TGG       251
Glu Glu Gly Ser Leu Val Val Gly Gly Tyr Tyr Pro Thr Glu Val Trp
     50                  55                  60

TAT AAC TGT TCC ACA ACT CAA CAA ACT ACC GCT TAT AAG TAT TTT AGT       299
Tyr Asn Cys Ser Thr Thr Gln Gln Thr Thr Ala Tyr Lys Tyr Phe Ser
 65                  70                  75                  80

AAT ATA CAT GCA TTT TAT TTT GAT ATG GAA GCC ATG GAG AAT AGT ACT       347
Asn Ile His Ala Phe Tyr Phe Asp Met Glu Ala Met Glu Asn Ser Thr
                 85                  90                  95

GGC AAT GCA CGT GGT AAA CCT TTA CTA GTA CAT GTT CAT GGT AAT CCT       395
Gly Asn Ala Arg Gly Lys Pro Leu Leu Val His Val His Gly Asn Pro
            100                 105                 110

GTT AGT ATC ATT GTT TAC ATA TCA GCT TAT AGA GAT GAT GTG CAA TTT       443
Val Ser Ile Ile Val Tyr Ile Ser Ala Tyr Arg Asp Asp Val Gln Phe
        115                 120                 125

AGG CCG CTT TTA AAG CAT GGT TTA TTG TGT ATA ACT AAA AAT GAC ACC       491
Arg Pro Leu Leu Lys His Gly Leu Leu Cys Ile Thr Lys Asn Asp Thr
    130                 135                 140

GTT GAC TAT AAT AGC TTT ACA ATT AAC CAA TGG CGA GAC ATA TGT TTG       539
Val Asp Tyr Asn Ser Phe Thr Ile Asn Gln Trp Arg Asp Ile Cys Leu
145                 150                 155                 160
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GGT GAC GAC AGA AAA ATA CCA TTC TCT GTA GTA CCC ACA GAT AAT GGT      587
Gly Asp Asp Arg Lys Ile Pro Phe Ser Val Val Pro Thr Asp Asn Gly
                165                 170                 175

ACG AAA TTA TTT GGT CTT GAG TGG AAT GAT GAC TAT GTT ACA GCC TAT      635
Thr Lys Leu Phe Gly Leu Glu Trp Asn Asp Asp Tyr Val Thr Ala Tyr
            180                 185                 190

ATT AGT GAT GAG TCT CAC CGT TTG AAT ATC AAT AAT AAT TGG TTT AAC      683
Ile Ser Asp Glu Ser His Arg Leu Asn Ile Asn Asn Asn Trp Phe Asn
        195                 200                 205

AAT GTT ACA CTC CTA TAC TCA CGT ACA AGC ACC GCC ACG TGG CAA CAC      731
Asn Val Thr Leu Leu Tyr Ser Arg Thr Ser Thr Ala Thr Trp Gln His
    210                 215                 220

AGT GCT GCA TAT GTT TAT CAA GGT GTT TCA AAT TTT ACT TAT TAC AAG      779
Ser Ala Ala Tyr Val Tyr Gln Gly Val Ser Asn Phe Thr Tyr Tyr Lys
225                 230                 235                 240

TTA AAT AAA ACC GCT GGC TTA AAA AGC TAT GAA TTG TGT GAA GAT TAT      827
Leu Asn Lys Thr Ala Gly Leu Lys Ser Tyr Glu Leu Cys Glu Asp Tyr
            245                 250                 255

GAA TAC TGC ACT GGC TAT GCA ACC AAT GTG TTT GCT CCG ACA TCA GGT      875
Glu Tyr Cys Thr Gly Tyr Ala Thr Asn Val Phe Ala Pro Thr Ser Gly
        260                 265                 270

GGT TAT ATA CCT GAT GGA TTC AGT TTT AAC AAT TGG TTT ATG CTT ACA      923
Gly Tyr Ile Pro Asp Gly Phe Ser Phe Asn Asn Trp Phe Met Leu Thr
    275                 280                 285

AAC AGC TCC ACT TTT GTT AGT GGC AGA TTT GTA ACA AAT CAA CCG CTG      971
Asn Ser Ser Thr Phe Val Ser Gly Arg Phe Val Thr Asn Gln Pro Leu
    290                 295                 300

CTA GTT AAT TGC TTG TGG CCA GTG CCC AGT TTT GGC GTC GCA GCA CAA     1019
Leu Val Asn Cys Leu Trp Pro Val Pro Ser Phe Gly Val Ala Ala Gln
305                 310                 315                 320

GAA TTT TGT TTT GAA GGT GCT CAG TTT AGC CAA TGT AAC GGT GTT TCT     1067
Glu Phe Cys Phe Glu Gly Ala Gln Phe Ser Gln Cys Asn Gly Val Ser
            325                 330                 335

TTA AAT AAT ACA GTA GAT GTT ATT AGA TTT AAC CTT AAT TTC ACT ACA     1115
Leu Asn Asn Thr Val Asp Val Ile Arg Phe Asn Leu Asn Phe Thr Thr
        340                 345                 350

GAT GTA CAA TCT GGC ATG GGT GCT ACA GTA TTT TCA CTG AAT ACA ACA     1163
Asp Val Gln Ser Gly Met Gly Ala Thr Val Phe Ser Leu Asn Thr Thr
    355                 360                 365

GGC GGT GTC ATT CTT GAG ATT TCT TGT TAT AAT GAC ACA GTG AGT GAG     1211
Gly Gly Val Ile Leu Glu Ile Ser Cys Tyr Asn Asp Thr Val Ser Glu
    370                 375                 380
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TCG AGT TTC TAC AGT TAT GGT GAA ATT CCA TTC GGC GTA ACT GAT GGA     1259
Ser Ser Phe Tyr Ser Tyr Gly Glu Ile Pro Phe Gly Val Thr Asp Gly
385                 390                 395                 400

CCA CGT TAC TGT TAT GTA CTC TAC AAT GGC ACA GCT CTT AAG TAT TTA     1307
Pro Arg Tyr Cys Tyr Val Leu Tyr Asn Gly Thr Ala Leu Lys Tyr Leu
            405                 410                 415

GGA ACA TTA CCA CCT AGT GTC AAG GAA ATT GCT ATT AGT AAG TGG GGA     1355
Gly Thr Leu Pro Pro Ser Val Lys Glu Ile Ala Ile Ser Lys Trp Gly
        420                 425                 430

CAT TTT TAT ATT AAT GGT TAC AAT TTC TTT AGC ACG TTT CCT ATT GAT     1403
His Phe Tyr Ile Asn Gly Tyr Asn Phe Phe Ser Thr Phe Pro Ile Asp
        435                 440                 445

TGT ATA GCT TTT AAT TTA ACC ACT GGT GCT AGT GGA GCA TTT TGG ACA     1451
Cys Ile Ala Phe Asn Leu Thr Thr Gly Ala Ser Gly Ala Phe Trp Thr
    450                 455                 460

ATT GCT TAT ACG TCG TAC ACA GAA GCA TTA GTA CAA GTT GAA AAC ACA     1499
Ile Ala Tyr Thr Ser Tyr Thr Glu Ala Leu Val Gln Val Glu Asn Thr
465                 470                 475                 480

GCT ATT AAA AAG GTG ACG TAT TGT AAC AGT CAC ATT AAT AAC ATC AAA     1547
Ala Ile Lys Lys Val Thr Tyr Cys Asn Ser His Ile Asn Asn Ile Lys
            485                 490                 495

TGT TCT CAA CTT ACT GCT AAT TTG CAA AAT GGT TTT TAC CCT GTT GCT     1595
Cys Ser Gln Leu Thr Ala Asn Leu Gln Asn Gly Phe Tyr Pro Val Ala
            500                 505                 510

TCA AGT GAA GTT GGT CTT GTC AAT AAG AGT GTT GTG TTA CTA CCT AGT     1643
Ser Ser Glu Val Gly Leu Val Asn Lys Ser Val Val Leu Leu Pro Ser
        515                 520                 525

TTC TAT TCA CAT ACC AGT GTT AAT ATA ACT ATT GAT CTT GGT ATG AAG     1691
Phe Tyr Ser His Thr Ser Val Asn Ile Thr Ile Asp Leu Gly Met Lys
    530                 535                 540

CGT AGT GTT ACG GTC ACC ATA GCC TCA CCA TTA AGT AAC ATC ACA CTA     1739
Arg Ser Val Thr Val Thr Ile Ala Ser Pro Leu Ser Asn Ile Thr Leu
545                 550                 555                 560

CCA ATG CAG GAT AAT AAC ATA GAC GTG TAC TGT ATT CGT TCT AAC CAA     1787
Pro Met Gln Asp Asn Asn Ile Asp Val Tyr Cys Ile Arg Ser Asn Gln
            565                 570                 575

TTC TCA GTT TAT GTT CAT TCC ACT TGC AAA AGT TCT TTA TGG GAT AAC     1835
Phe Ser Val Tyr Val His Ser Thr Cys Lys Ser Ser Leu Trp Asp Asn
        580                 585                 590

AAT TTT AAT TCA GCA TGT ACC GAC GTT TTA GAC GCC ACA GCT GTT ATA     1883
Asn Phe Asn Ser Ala Cys Thr Asp Val Leu Asp Ala Thr Ala Val Ile
        595                 600                 605
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AAA ACT GGT ACT TGT CCT TTC TCA TTT GAT AAA TTG AAT AAT TAC TTA     1931
Lys Thr Gly Thr Cys Pro Phe Ser Phe Asp Lys Leu Asn Asn Tyr Leu
    610                 615                 620

ACT TTT AAC AAG TTC TGT TTG TCG TTG AAT CCC GTT GGT GCC AAC TGT     1979
Thr Phe Asn Lys Phe Cys Leu Ser Leu Asn Pro Val Gly Ala Asn Cys
625                 630                 635                 640

AAG TTA GAT GTT GCC GCC CGT ACA AGA ACC AAT GAG CAG GTT TTT GGA     2027
Lys Leu Asp Val Ala Ala Arg Thr Arg Thr Asn Glu Gln Val Phe Gly
                645                 650                 655

AGT TTA TAT GTA ATA TAT GAA GAA GGA GAC AAC ATA GTG GGT GTA CCG     2075
Ser Leu Tyr Val Ile Tyr Glu Glu Gly Asp Asn Ile Val Gly Val Pro
            660                 665                 670

TCT GAT AAT AGT GGT TTG CAC GAT TTG TCA GTG TTG CAC TTA GAC TCT     2123
Ser Asp Asn Ser Gly Leu His Asp Leu Ser Val Leu His Leu Asp Ser
        675                 680                 685

TGT ACA GAT TAC AAT ATA TAT GGT AGA ACT GGT GTT GGT ATT ATT AGA     2171
Cys Thr Asp Tyr Asn Ile Tyr Gly Arg Thr Gly Val Gly Ile Ile Arg
    690                 695                 700

AAA ACT AAC AGC ACA CTA CTT AGT GGC TTA TAT TAC ACA TCA CTA TCA     2219
Lys Thr Asn Ser Thr Leu Leu Ser Gly Leu Tyr Tyr Thr Ser Leu Ser
705                 710                 715                 720

GGT GAT TTG TTA GGT TTT AAA AAT GTT AGT GAT GGT GTT GTC TAC TCT     2267
Gly Asp Leu Leu Gly Phe Lys Asn Val Ser Asp Gly Val Val Tyr Ser
                725                 730                 735

GTA ACG CCA TGT GAT GTA AGT GCA CAA GCT GCT GTT ATT GAT GGT GCC     2315
Val Thr Pro Cys Asp Val Ser Ala Gln Ala Ala Val Ile Asp Gly Ala
            740                 745                 750

ATA GTT GGA GCT ATG ACT TCC ATT AAT AGT GAA CTG TTA GGT CTA ACT     2363
Ile Val Gly Ala Met Thr Ser Ile Asn Ser Glu Leu Leu Gly Leu Thr
        755                 760                 765

CAT TGG ACA ACA ACA CCT AAT TTT TAT TAC TAC TCC ATA TAT AAT TAT     2411
His Trp Thr Thr Thr Pro Asn Phe Tyr Tyr Tyr Ser Ile Tyr Asn Tyr
    770                 775                 780

ACA AAT GTG ATG AAT CGT GGC ACG GCA ATT GAT AAT GAT ATT GAT TGT     2459
Thr Asn Val Met Asn Arg Gly Thr Ala Ile Asp Asn Asp Ile Asp Cys
785                 790                 795                 800

GAA CCT ATC ATA ACA TAT TCT AAT ATA GGT GTT TGT AAA AAT GGA GCT     2507
Glu Pro Ile Ile Thr Tyr Ser Asn Ile Gly Val Cys Lys Asn Gly Ala
                805                 810                 815

TTG GTT TTT ATT AAC GTC ACA CAT TCT GAT GGA GAC GTT CAA CCA ATT     2555
Leu Val Phe Ile Asn Val Thr His Ser Asp Gly Asp Val Gln Pro Ile
            820                 825                 830
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AGC ACC GGT AAT GTC ACG ATA CCC ACA AAT TTT ACT ATA TCT GTG CAA     2603
Ser Thr Gly Asn Val Thr Ile Pro Thr Asn Phe Thr Ile Ser Val Gln
        835                     840                     845

GTC GAA TAT ATT CAG GTT TAC ACT ACA CCA GTT TCA ATA GAC TGT GCA     2651
Val Glu Tyr Ile Gln Val Tyr Thr Thr Pro Val Ser Ile Asp Cys Ala
    850                     855                     860

AGA TAC GTT TGC AAT GGT AAC CCA AGA TGC AAT AAG TTA TTA ACA CAA     2699
Arg Tyr Val Cys Asn Gly Asn Pro Arg Cys Asn Lys Leu Leu Thr Gln
865                     870                     875                 880

TAC GTT TCT GCA TGT CAA ACT ATT GAG CAA GCG CTT GCA ATG GGT GCC     2747
Tyr Val Ser Ala Cys Gln Thr Ile Glu Gln Ala Leu Ala Met Gly Ala
                885                     890                     895

AGA CTT GAA AAC ATG GAG ATT GAT TCC ATG TTA TTT GTT TCG GAA AAT     2795
Arg Leu Glu Asn Met Glu Ile Asp Ser Met Leu Phe Val Ser Glu Asn
            900                     905                     910

GCC CTT AAA TTG GCA TCT GTT GAA GCA TTC AAT AGT ACG GAA AAT TTA     2843
Ala Leu Lys Leu Ala Ser Val Glu Ala Phe Asn Ser Thr Glu Asn Leu
        915                     920                     925

GAC CCT ATT TAT AAA GAA TGG CCT AAC ATT GGT GGT TCT TGG CTA GGA     2891
Asp Pro Ile Tyr Lys Glu Trp Pro Asn Ile Gly Gly Ser Trp Leu Gly
    930                     935                     940

GGT TTA AAA GAT ATA TTG CCA TCT CAT AAT AGC AAA CGT AAG TAC CGC     2939
Gly Leu Lys Asp Ile Leu Pro Ser His Asn Ser Lys Arg Lys Tyr Arg
945                     950                     955                 960

TCG GCT ATA GAA GAC TTG CTT TTT GAT AAG GTT GTA ACA TCT GGC TTA     2987
Ser Ala Ile Glu Asp Leu Leu Phe Asp Lys Val Val Thr Ser Gly Leu
                965                     970                     975

GGT ACA GTT GAC GAA GAT TAC AAA CGT TCT GCA GGT GGT TAT GAC ATA     3035
Gly Thr Val Asp Glu Asp Tyr Lys Arg Ser Ala Gly Gly Tyr Asp Ile
            980                     985                     990

GCT GAC TTA GTG TGT GCA CGA TAT TAC AAT GGC ATC ATG GTG CTA CCT     3083
Ala Asp Leu Val Cys Ala Arg Tyr Tyr Asn Gly Ile Met Val Leu Pro
        995                     1000                    1005

GGT GTA GCT AAT GAT GAC AAG ATG ACT ATG TAC ACT GCA TCT CTT ACA     3131
Gly Val Ala Asn Asp Asp Lys Met Thr Met Tyr Thr Ala Ser Leu Thr
    1010                    1015                    1020

GGT GGT ATA ACA TTA GGT GCA CTT AGT GGT GGC GCA GTG GCT ATA CCT     3179
Gly Gly Ile Thr Leu Gly Ala Leu Ser Gly Gly Ala Val Ala Ile Pro
1025                    1030                    1035                1040

TTT GCA GTA GCA GTT CAG GCT AGA CTT AAT TAT GTT GCT CTA CAA ACT     3227
Phe Ala Val Ala Val Gln Ala Arg Leu Asn Tyr Val Ala Leu Gln Thr
                1045                    1050                    1055
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GAT GTA TTG AAC AAA AAC CAA CAA ATC TTG GCT AAT GCT TTC AAT CAA      3275
Asp Val Leu Asn Lys Asn Gln Gln Ile Leu Ala Asn Ala Phe Asn Gln
            1060                1065                1070

GCT ATT GGT AAC ATT ACA CAG GCA TTT GGT AAG GTT AAT GAC GCT ATA      3323
Ala Ile Gly Asn Ile Thr Gln Ala Phe Gly Lys Val Asn Asp Ala Ile
        1075                1080                1085

CAT CAA ACA TCA AAA GGT CTT GCT ACT GTT GCT AAA GCA TTG GCA AAG      3371
His Gln Thr Ser Lys Gly Leu Ala Thr Val Ala Lys Ala Leu Ala Lys
    1090                1095                1100

GTG CAA GAT GTT GTT AAC ACG CAA GGT CAA GCT TTA AGC CAC CTA ACA      3419
Val Gln Asp Val Val Asn Thr Gln Gly Gln Ala Leu Ser His Leu Thr
1105                1110                1115                1120

GTA CAA TTG CAA AAC AAT TTT CAA GCC ATT AGC AGT TCT ATT AGT GAC      3467
Val Gln Leu Gln Asn Asn Phe Gln Ala Ile Ser Ser Ser Ile Ser Asp
                1125                1130                1135

ATT TAT AAC AGG CTT GAT GAA TTG AGT GCT GAT GCA CAA GTT GAC AGG      3515
Ile Tyr Asn Arg Leu Asp Glu Leu Ser Ala Asp Ala Gln Val Asp Arg
            1140                1145                1150

CTG ATT ACA GGA CGA CTT ACA GCA CTT AAT GCA TTT GTG TCT CAG ACT      3563
Leu Ile Thr Gly Arg Leu Thr Ala Leu Asn Ala Phe Val Ser Gln Thr
        1155                1160                1165

TTA ACC AGA CAA GCA GAG GTT AGG GCT AGT AGA CAA CTT GCT AAA GAC      3611
Leu Thr Arg Gln Ala Glu Val Arg Ala Ser Arg Gln Leu Ala Lys Asp
    1170                1175                1180

AAG GTT AAT GAA TGC GTT AGG TCT CAA TCC CAG AGA TTT GGA TTC TGT      3659
Lys Val Asn Glu Cys Val Arg Ser Gln Ser Gln Arg Phe Gly Phe Cys
1185                1190                1195                1200

GGT AAT GGT ACA CAT TTG TTT TCA CTT GCA AAT GCG GCA CCA AAT GGC      3707
Gly Asn Gly Thr His Leu Phe Ser Leu Ala Asn Ala Ala Pro Asn Gly
                1205                1210                1215

ATG ATT TTC TTT CAC ACA GTG CTA TTA CCA ACA GCT TAT GAA ACT GTG      3755
Met Ile Phe Phe His Thr Val Leu Leu Pro Thr Ala Tyr Glu Thr Val
            1220                1225                1230

ACG GCC TGG TCA GGT ATT TGT GCG TCA GAT GGC AGT CGC ACT TTT GGA      3803
Thr Ala Trp Ser Gly Ile Cys Ala Ser Asp Gly Ser Arg Thr Phe Gly
        1235                1240                1245

CTT GTT GTT GAG GAT GTC CAG CTG ACG CTA TTT CGC AAT TTA GAT GAA      3851
Leu Val Val Glu Asp Val Gln Leu Thr Leu Phe Arg Asn Leu Asp Glu
    1250                1255                1260

AAA TTT TAT TTG ACG CCC AGA ACT ATG TAT CAG CCC AGA GTT GCA ACT      3899
Lys Phe Tyr Leu Thr Pro Arg Thr Met Tyr Gln Pro Arg Val Ala Thr
1265                1270                1275                1280
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AGT TCT GAT TTT GTT CAA ATA GAA GGC TGT GAT GTG TTG TTT GTT AAT      3947
Ser Ser Asp Phe Val Gln Ile Glu Gly Cys Asp Val Leu Phe Val Asn
                1285                1290                1295

GGA ACT GTA ATT GAA TTG CCT AGT ATC ATA CCT GAC TAT ATC GAT ATT      3995
Gly Thr Val Ile Glu Leu Pro Ser Ile Ile Pro Asp Tyr Ile Asp Ile
            1300                1305                1310

AAT CAA ACT GTT CAG GAC ATA TTA GAA AAT TTC AGA CCA AAT TGG ACT      4043
Asn Gln Thr Val Gln Asp Ile Leu Glu Asn Phe Arg Pro Asn Trp Thr
        1315                1320                1325

GTA CCC GAG TTG CCA CTT GAC ATT TTT CAT GCA ACC TAC TTA AAC CTG      4091
Val Pro Glu Leu Pro Leu Asp Ile Phe His Ala Thr Tyr Leu Asn Leu
    1330                1335                1340

ACT GGT GAA ATT AAT GAC TTA GAA TTT AGG TCA GAA AAG TTA CAT AAC      4139
Thr Gly Glu Ile Asn Asp Leu Glu Phe Arg Ser Glu Lys Leu His Asn
1345                1350                1355                1360

ACC ACA GTA GAA CTT GCT ATT CTC ATT GAT AAT ATT AAT AAC ACA TTA      4187
Thr Thr Val Glu Leu Ala Ile Leu Ile Asp Asn Ile Asn Asn Thr Leu
                1365                1370                1375

GTC AAT CTT GAA TGG CTC AAC AGA ATT GAA ACT TAT GTA AAA TGG CCT      4235
Val Asn Leu Glu Trp Leu Asn Arg Ile Glu Thr Tyr Val Lys Trp Pro
            1380                1385                1390

TGG TAT GTT TGG CTA CTA ATT GGA TTA GTA GTA ATA TTC TGC ATA CCC      4283
Trp Tyr Val Trp Leu Leu Ile Gly Leu Val Val Ile Phe Cys Ile Pro
        1395                1400                1405

ATA TTG CTA TTT TGT TGT TGT AGT ACT GGT TGT TGT GGA TGT ATC GGG      4331
Ile Leu Leu Phe Cys Cys Cys Ser Thr Gly Cys Cys Gly Cys Ile Gly
    1410                1415                1420

TGT TTA GGA AGC TGT TGT CAT TCC ATA TGT AGT AGA GGC CAA TTT GAA      4379
Cys Leu Gly Ser Cys Cys His Ser Ile Cys Ser Arg Gly Gln Phe Glu
1425                1430                1435                1440

AGT TAT GAA CCT ATT GAA AAA GTT CAT GTT CAC TGAATTCAAA ATGTTAA       4429
Ser Tyr Glu Pro Ile Glu Lys Val His Val His
                1445                1450
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1451 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Canine corona virus
(B) STRAIN: CCVInSAVC-1

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..1451
(D) OTHER INFORMATION: /label= CCVInSAVC-1_Spike

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Ile Val Leu Thr Leu Cys Leu Phe Leu Phe Leu Tyr Ser Ser Val
1               5                   10                  15

Ser Cys Thr Ser Asn Asn Asp Cys Val Gln Val Asn Val Thr Gln Leu
            20                  25                  30

Pro Gly Asn Glu Asn Ile Ile Lys Asp Phe Leu Phe Gln Asn Phe Lys
        35                  40                  45

Glu Glu Gly Ser Leu Val Val Gly Gly Tyr Tyr Pro Thr Glu Val Trp
    50                  55                  60

Tyr Asn Cys Ser Thr Thr Gln Gln Thr Thr Ala Tyr Lys Tyr Phe Ser
65                  70                  75                  80

Asn Ile His Ala Phe Tyr Phe Asp Met Glu Ala Met Glu Asn Ser Thr
                85                  90                  95

Gly Asn Ala Arg Gly Lys Pro Leu Leu Val His Val His Gly Asn Pro
            100                 105                 110

Val Ser Ile Ile Val Tyr Ile Ser Ala Tyr Arg Asp Asp Val Gln Phe
        115                 120                 125

Arg Pro Leu Leu Lys His Gly Leu Leu Cys Ile Thr Lys Asn Asp Thr
    130                 135                 140

Val Asp Tyr Asn Ser Phe Thr Ile Asn Gln Trp Arg Asp Ile Cys Leu
145                 150                 155                 160

Gly Asp Asp Arg Lys Ile Pro Phe Ser Val Val Pro Thr Asp Asn Gly
                165                 170                 175

Thr Lys Leu Phe Gly Leu Glu Trp Asn Asp Asp Tyr Val Thr Ala Tyr
            180                 185                 190

Ile Ser Asp Glu Ser His Arg Leu Asn Ile Asn Asn Asn Trp Phe Asn
        195                 200                 205

Asn Val Thr Leu Leu Tyr Ser Arg Thr Ser Thr Ala Thr Trp Gln His
    210                 215                 220
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Ser Ala Ala Tyr Val Tyr Gln Gly Val Ser Asn Phe Thr Tyr Tyr Lys
225                 230                 235                 240

Leu Asn Lys Thr Ala Gly Leu Lys Ser Tyr Glu Leu Cys Glu Asp Tyr
                245                 250                 255

Glu Tyr Cys Thr Gly Tyr Ala Thr Asn Val Phe Ala Pro Thr Ser Gly
            260                 265                 270

Gly Tyr Ile Pro Asp Gly Phe Ser Phe Asn Asn Trp Phe Met Leu Thr
        275                 280                 285

Asn Ser Ser Thr Phe Val Ser Gly Arg Phe Val Thr Asn Gln Pro Leu
    290                 295                 300

Leu Val Asn Cys Leu Trp Pro Val Pro Ser Phe Gly Val Ala Ala Gln
305                 310                 315                 320

Glu Phe Cys Phe Glu Gly Ala Gln Phe Ser Gln Cys Asn Gly Val Ser
                325                 330                 335

Leu Asn Asn Thr Val Asp Val Ile Arg Phe Asn Leu Asn Phe Thr Thr
            340                 345                 350

Asp Val Gln Ser Gly Met Gly Ala Thr Val Phe Ser Leu Asn Thr Thr
        355                 360                 365

Gly Gly Val Ile Leu Glu Ile Ser Cys Tyr Asn Asp Thr Val Ser Glu
    370                 375                 380

Ser Ser Phe Tyr Ser Tyr Gly Glu Ile Pro Phe Gly Val Thr Asp Gly
385                 390                 395                 400

Pro Arg Tyr Cys Tyr Val Leu Tyr Asn Gly Thr Ala Leu Lys Tyr Leu
                405                 410                 415

Gly Thr Leu Pro Pro Ser Val Lys Glu Ile Ala Ile Ser Lys Trp Gly
            420                 425                 430

His Phe Tyr Ile Asn Gly Tyr Asn Phe Phe Ser Thr Phe Pro Ile Asp
        435                 440                 445

Cys Ile Ala Phe Asn Leu Thr Thr Gly Ala Ser Gly Ala Phe Trp Thr
    450                 455                 460

Ile Ala Tyr Thr Ser Tyr Thr Glu Ala Leu Val Gln Val Glu Asn Thr
465                 470                 475                 480

Ala Ile Lys Lys Val Thr Tyr Cys Asn Ser His Ile Asn Asn Ile Lys
                485                 490                 495

Cys Ser Gln Leu Thr Ala Asn Leu Gln Asn Gly Phe Tyr Pro Val Ala
            500                 505                 510

Ser Ser Glu Val Gly Leu Val Asn Lys Ser Val Val Leu Leu Pro Ser
        515                 520                 525
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Phe Tyr Ser His Thr Ser Val Asn Ile Thr Ile Asp Leu Gly Met Lys
    530                 535                 540

Arg Ser Val Thr Val Thr Ile Ala Ser Pro Leu Ser Asn Ile Thr Leu
545                 550                 555                 560

Pro Met Gln Asp Asn Asn Ile Asp Val Tyr Cys Ile Arg Ser Asn Gln
                565                 570                 575

Phe Ser Val Tyr Val His Ser Thr Cys Lys Ser Ser Leu Trp Asp Asn
            580                 585                 590

Asn Phe Asn Ser Ala Cys Thr Asp Val Leu Asp Ala Thr Ala Val Ile
        595                 600                 605

Lys Thr Gly Thr Cys Pro Phe Ser Phe Asp Lys Leu Asn Asn Tyr Leu
    610                 615                 620

Thr Phe Asn Lys Phe Cys Leu Ser Leu Asn Pro Val Gly Ala Asn Cys
625                 630                 635                 640

Lys Leu Asp Val Ala Ala Arg Thr Arg Thr Asn Glu Gln Val Phe Gly
                645                 650                 655

Ser Leu Tyr Val Ile Tyr Glu Glu Gly Asp Asn Ile Val Gly Val Pro
            660                 665                 670

Ser Asp Asn Ser Gly Leu His Asp Leu Ser Val Leu His Leu Asp Ser
        675                 680                 685

Cys Thr Asp Tyr Asn Ile Tyr Gly Arg Thr Gly Val Gly Ile Ile Arg
    690                 695                 700

Lys Thr Asn Ser Thr Leu Leu Ser Gly Leu Tyr Tyr Thr Ser Leu Ser
705                 710                 715                 720

Gly Asp Leu Leu Gly Phe Lys Asn Val Ser Asp Gly Val Val Tyr Ser
                725                 730                 735

Val Thr Pro Cys Asp Val Ser Ala Gln Ala Ala Val Ile Asp Gly Ala
            740                 745                 750

Ile Val Gly Ala Met Thr Ser Ile Asn Ser Glu Leu Leu Gly Leu Thr
        755                 760                 765

His Trp Thr Thr Thr Pro Asn Phe Tyr Tyr Tyr Ser Ile Tyr Asn Tyr
    770                 775                 780

Thr Asn Val Met Asn Arg Gly Thr Ala Ile Asp Asn Asp Ile Asp Cys
785                 790                 795                 800

Glu Pro Ile Ile Thr Tyr Ser Asn Ile Gly Val Cys Lys Asn Gly Ala
                805                 810                 815
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Leu Val Phe Ile Asn Val Thr His Ser Asp Gly Asp Val Gln Pro Ile
820 825 830

Ser Thr Gly Asn Val Thr Ile Pro Thr Asn Phe Thr Ile Ser Val Gln
835 840 845

Val Glu Tyr Ile Gln Val Tyr Thr Thr Pro Val Ser Ile Asp Cys Ala
850 855 860

Arg Tyr Val Cys Asn Gly Asn Pro Arg Cys Asn Lys Leu Leu Thr Gln
865 870 875 880

Tyr Val Ser Ala Cys Gln Thr Ile Glu Gln Ala Leu Ala Met Gly Ala
885 890 895

Arg Leu Glu Asn Met Glu Ile Asp Ser Met Leu Phe Val Ser Glu Asn
900 905 910

Ala Leu Lys Leu Ala Ser Val Glu Ala Phe Asn Ser Thr Glu Asn Leu
915 920 925

Asp Pro Ile Tyr Lys Glu Trp Pro Asn Ile Gly Gly Ser Trp Leu Gly
930 935 940

Gly Leu Lys Asp Ile Leu Pro Ser His Asn Ser Lys Arg Lys Tyr Arg
945 950 955 960

Ser Ala Ile Glu Asp Leu Leu Phe Asp Lys Val Val Thr Ser Gly Leu
965 970 975

Gly Thr Val Asp Glu Asp Tyr Lys Arg Ser Ala Gly Gly Tyr Asp Ile
980 985 990

Ala Asp Leu Val Cys Ala Arg Tyr Tyr Asn Gly Ile Met Val Leu Pro
995 1000 1005

Gly Val Ala Asn Asp Asp Lys Met Thr Met Tyr Thr Ala Ser Leu Thr
1010 1015 1020

Gly Gly Ile Thr Leu Gly Ala Leu Ser Gly Gly Ala Val Ala Ile Pro
1025 1030 1035 104

Phe Ala Val Ala Val Gln Ala Arg Leu Asn Tyr Val Ala Leu Gln Thr
1045 1050 1055

Asp Val Leu Asn Lys Asn Gln Gln Ile Leu Ala Asn Ala Phe Asn Gln
1060 1065 1070

Ala Ile Gly Asn Ile Thr Gln Ala Phe Gly Lys Val Asn Asp Ala Ile
1075 1080 1085

His Gln Thr Ser Lys Gly Leu Ala Thr Val Ala Lys Ala Leu Ala Lys
1090 1095 1100

Val Gln Asp Val Val Asn Thr Gln Gly Gln Ala Leu Ser His Leu Thr
1105 1110 1115 112

Val Gln Leu Gln Asn Asn Phe Gln Ala Ile Ser Ser Ser Ile Ser Asp
1125 1130 1135

Ile Tyr Asn Arg Leu Asp Glu Leu Ser Ala Asp Ala Gln Val Asp Arg
1140 1145 1150

Leu Ile Thr Gly Arg Leu Thr Ala Leu Asn Ala Phe Val Ser Gln Thr
1155 1160 1165

Leu Thr Arg Gln Ala Glu Val Arg Ala Ser Arg Gln Leu Ala Lys Asp
1170 1175 1180

Lys Val Asn Glu Cys Val Arg Ser Gln Ser Gln Arg Phe Gly Phe Cys
1185 1190 1195 120

Gly Asn Gly Thr His Leu Phe Ser Leu Ala Asn Ala Ala Pro Asn Gly
1205 1210 1215

Met Ile Phe Phe His Thr Val Leu Leu Pro Thr Ala Tyr Glu Thr Val
1220 1225 1230

Thr Ala Trp Ser Gly Ile Cys Ala Ser Asp Gly Ser Arg Thr Phe Gly
1235 1240 1245

Leu Val Val Glu Asp Val Gln Leu Thr Leu Phe Arg Asn Leu Asp Glu
1250 1255 1260

Lys Phe Tyr Leu Thr Pro Arg Thr Met Tyr Gln Pro Arg Val Ala Thr
1265 1270 1275 128

Ser Ser Asp Phe Val Gln Ile Glu Gly Cys Asp Val Leu Phe Val Asn
1285 1290 1295

Gly Thr Val Ile Glu Leu Pro Ser Ile Ile Pro Asp Tyr Ile Asp Ile
1300 1305 1310

Asn Gln Thr Val Gln Asp Ile Leu Glu Asn Phe Arg Pro Asn Trp Thr
1315 1320 1325

Val Pro Glu Leu Pro Leu Asp Ile Phe His Ala Thr Tyr Leu Asn Leu
1330 1335 1340

Thr Gly Glu Ile Asn Asp Leu Glu Phe Arg Ser Glu Lys Leu His Asn
1345 1350 1355 136

Thr Thr Val Glu Leu Ala Ile Leu Ile Asp Asn Ile Asn Asn Thr Leu
1365 1370 1375

Val Asn Leu Glu Trp Leu Asn Arg Ile Glu Thr Tyr Val Lys Trp Pro
1380 1385 1390

Trp Tyr Val Trp Leu Leu Ile Gly Leu Val Val Ile Phe Cys Ile Pro
1395 1400 1405

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Ile Leu Leu Phe Cys Cys Cys Ser Thr Gly Cys Cys Gly Cys Ile Gly
    1410                1415                1420

Cys Leu Gly Ser Cys Cys His Ser Ile Cys Ser Arg Gly Gln Phe Glu
1425                1430                1435                144

Ser Tyr Glu Pro Ile Glu Lys Val His Val His
                1445                1450
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4435 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Canine corona virus
(B) STRAIN: CCV-V54

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 60..4418
(D) OTHER INFORMATION: /label= CCV-C54_Spikegene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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TTGCTCATTA GAAACAATGG AAAACTACTA AACTTCGGTA ATCACTTGGT TAATGTGCC        59

ATG ATT GTG CTT ACA TTG TGC CTT CTC TTG TTT TCA TAC AAT AGT GTG        107
Met Ile Val Leu Thr Leu Cys Leu Leu Leu Phe Ser Tyr Asn Ser Val
 1               5                  10                  15

ATT TGT ACA TCA AAT AAT GAT TGT GTA CAA GTT AAT GTG ACA CAA TTG        155
Ile Cys Thr Ser Asn Asn Asp Cys Val Gln Val Asn Val Thr Gln Leu
            20                  25                  30

CCT GGC AAT GAA AAT ATC ATT AAA GAT TTT CTA TTT CAG AAT TTT AAA        203
Pro Gly Asn Glu Asn Ile Ile Lys Asp Phe Leu Phe Gln Asn Phe Lys
        35                  40                  45

GAA GAA GGA AGT GTA GTT GTT GGT GGC TAC TAC CCC ACA GAG GTG TGG        251
Glu Glu Gly Ser Val Val Val Gly Gly Tyr Tyr Pro Thr Glu Val Trp
    50                  55                  60

TAC AAC TGT TCC AGA ACA GCA ACA ACT ACA GCT TAC CAT TAT TTT AGT        299
Tyr Asn Cys Ser Arg Thr Ala Thr Thr Thr Ala Tyr His Tyr Phe Ser
65                  70                  75                  80
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AAC ATA CAT GCA TTT TAT TTT GAT ATG GAA GCT ATG GCG AAT AGT ACT 347
Asn Ile His Ala Phe Tyr Phe Asp Met Glu Ala Met Ala Asn Ser Thr
85 90 95

GGC AAT GCA AGA GGT AAA CCT TTA CTA GTA CAT GTT CAT GGT AGT CCT 395
Gly Asn Ala Arg Gly Lys Pro Leu Leu Val His Val His Gly Ser Pro
100 105 110

GTT AGT ATC ATT GTT TAC ATA TCA GCC TAT AGA GAT GAT GTG CAA AAT 443
Val Ser Ile Ile Val Tyr Ile Ser Ala Tyr Arg Asp Asp Val Gln Asn
115 120 125

AGG CCG CTC TTA AAA CAT GGT TTG TTG TGT ATA ACT AAA AAC AGC ACC 491
Arg Pro Leu Leu Lys His Gly Leu Leu Cys Ile Thr Lys Asn Ser Thr
130 135 140

ATT GAT TAT AAC AGT TTT ACC TCT GCT CAG TGG CGT GAC ATA TGT TTG 539
Ile Asp Tyr Asn Ser Phe Thr Ser Ala Gln Trp Arg Asp Ile Cys Leu
145 150 155 160

GGT ACT GAC AGA AAA ATA CCA TTC TCC GTC GTA CCC ACA GAT AAT GGC 587
Gly Thr Asp Arg Lys Ile Pro Phe Ser Val Val Pro Thr Asp Asn Gly
165 170 175

ACA AAA CTA TTT GGT CTT GAG TGG ACT GAT GAC TAT GTT ACA GCC TAT 635
Thr Lys Leu Phe Gly Leu Glu Trp Thr Asp Asp Tyr Val Thr Ala Tyr
180 185 190

ATT AGT GAT GAT TCC CAC CGT TTG AAT ATC AAT ACT AAT TGG TTT AAC 683
Ile Ser Asp Asp Ser His Arg Leu Asn Ile Asn Thr Asn Trp Phe Asn
195 200 205

AAT GTT ACA ATC CTA TAC TCC CGC TCA AGT ACT GCC ACG TGG CAA AAG 731
Asn Val Thr Ile Leu Tyr Ser Arg Ser Ser Thr Ala Thr Trp Gln Lys
210 215 220

AGT GCC GCA TAT GTT TAT CAA GGT GTT TCA AAT TTT ACG TAT TAT AAG 779
Ser Ala Ala Tyr Val Tyr Gln Gly Val Ser Asn Phe Thr Tyr Tyr Lys
225 230 235 240

TTA AAC AAC ACC AAT GGC TTA AAA AGC TAT GAA TTG TGT GAA GAT TAT 827
Leu Asn Asn Thr Asn Gly Leu Lys Ser Tyr Glu Leu Cys Glu Asp Tyr
245 250 255

GAA TAC TGC ACT GGC TAT GCC ACC AAT GTG TTT GCT CCG ACA TCA GGT 875
Glu Tyr Cys Thr Gly Tyr Ala Thr Asn Val Phe Ala Pro Thr Ser Gly
260 265 270

GGT TAC ATA CCT GAT GGA TTC AGT TTT AAC AAT TGG TTT ATG CTT ACA 923
Gly Tyr Ile Pro Asp Gly Phe Ser Phe Asn Asn Trp Phe Met Leu Thr
275 280 285

AAC AGC TCC ACT TTT GTT AGT GGT AGG TTT GTA ACA AAT CAA CCG CTG 971
Asn Ser Ser Thr Phe Val Ser Gly Arg Phe Val Thr Asn Gln Pro Leu
290 295 300

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TTA GTT AAT TGC TTG GTG CCA GTG CCC AGT TTT GGT GTT GCA GCA CAA      1019
Leu Val Asn Cys Leu Val Pro Val Pro Ser Phe Gly Val Ala Ala Gln
305                 310                 315                 320

GAA TTT TGT TTT GAA GGT GCG CAG TTT AGC CAA TGT AAC GGT GTT TCT      1067
Glu Phe Cys Phe Glu Gly Ala Gln Phe Ser Gln Cys Asn Gly Val Ser
                325                 330                 335

TTA AAT AAC ACA GTA GAT GTC ATT AGA TTT AAC CTT AAT TTT ACT ACA      1115
Leu Asn Asn Thr Val Asp Val Ile Arg Phe Asn Leu Asn Phe Thr Thr
            340                 345                 350

AAT GTA CAA TCT GGC ATG GGT GCT ACA GTA TTT TCA CTG AAT ACA ACA      1163
Asn Val Gln Ser Gly Met Gly Ala Thr Val Phe Ser Leu Asn Thr Thr
        355                 360                 365

GGT GGT GTC ATT CTT GAG ATT TCT TGT TAT AAT GAT ACA GTG AGT GAG      1211
Gly Gly Val Ile Leu Glu Ile Ser Cys Tyr Asn Asp Thr Val Ser Glu
    370                 375                 380

TCG AGT TTC TAC AGT TAT GGT GAA ATT CCA TTC GGC GTA ACT GAT GGA      1259
Ser Ser Phe Tyr Ser Tyr Gly Glu Ile Pro Phe Gly Val Thr Asp Gly
385                 390                 395                 400

CCG CGT TAC TGT TAT GTA CTC TAT AAT GGC ACG GCT CTT AAG TAT TTA      1307
Pro Arg Tyr Cys Tyr Val Leu Tyr Asn Gly Thr Ala Leu Lys Tyr Leu
                405                 410                 415

GGA ACA TTA CCA CCT AGT GTC AAG GAA ATT GCT ATT AGT AAG TGG GGC      1355
Gly Thr Leu Pro Pro Ser Val Lys Glu Ile Ala Ile Ser Lys Trp Gly
            420                 425                 430

CAT TTT TAT ATT AAT GGT TAC AAT TTC TTT AGC ACT TTT CCT ATT GAT      1403
His Phe Tyr Ile Asn Gly Tyr Asn Phe Phe Ser Thr Phe Pro Ile Asp
        435                 440                 445

TGT ATA TCT TTT AAT TTA ACC ACT GGT GAT AGT GGA GCA TTT TGG ACA      1451
Cys Ile Ser Phe Asn Leu Thr Thr Gly Asp Ser Gly Ala Phe Trp Thr
    450                 455                 460

ATT GCT TAC ACA TCG TAC ACT GAA GCA TTA GTA CAA GTT GAA AAC ACA      1499
Ile Ala Tyr Thr Ser Tyr Thr Glu Ala Leu Val Gln Val Glu Asn Thr
465                 470                 475                 480

GCT ATT AAA AAG GTG ACG TAT TGT AAC AGT CAC ATT AAT AAC ATC AAA      1547
Ala Ile Lys Lys Val Thr Tyr Cys Asn Ser His Ile Asn Asn Ile Lys
                485                 490                 495

TGT TCT CAA CTT ACT GCT AAC TTG CAA AAT GGA TTT TAT CCT GTT GCT      1595
Cys Ser Gln Leu Thr Ala Asn Leu Gln Asn Gly Phe Tyr Pro Val Ala
            500                 505                 510

TCA AGT GAA GTT GGT CTT GTC AAT AAG AGT GTT GTG TTA CTA CCT AGT      1643
Ser Ser Glu Val Gly Leu Val Asn Lys Ser Val Val Leu Leu Pro Ser
        515                 520                 525
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TTC TAT TCA CAT ACC AGT GTT AAT ATA ACT ATT GAT CTT GGT ATG AAG      1691
Phe Tyr Ser His Thr Ser Val Asn Ile Thr Ile Asp Leu Gly Met Lys
    530                 535                     540

CGT AGT GGT TAT GGT CAA CCC ATA GCA TCA ACA CTA AGT AAC ATC ACA      1739
Arg Ser Gly Tyr Gly Gln Pro Ile Ala Ser Thr Leu Ser Asn Ile Thr
545                 550                     555                 560

CTA CCA ATG CAG GAT AAT AAC ACC GAT GTG TAC TGT ATT CGT TCC AAC      1787
Leu Pro Met Gln Asp Asn Asn Thr Asp Val Tyr Cys Ile Arg Ser Asn
                565                     570                 575

CAA TTT TCA GTC TAC GTG CAT TCC ACT TGC AAA AGC TCT TTA TGG GAC      1835
Gln Phe Ser Val Tyr Val His Ser Thr Cys Lys Ser Ser Leu Trp Asp
            580                     585                     590

AAT ATT TTT AAT TCA GAC TGT ACA GAT GTT TTA CAT GCC ACA GCT GTT      1883
Asn Ile Phe Asn Ser Asp Cys Thr Asp Val Leu His Ala Thr Ala Val
        595                     600                     605

ATA AAA ACT GGT ACT TGT CCT TTT TCA TTT GAT AAA TTG AAT AAT TAC      1931
Ile Lys Thr Gly Thr Cys Pro Phe Ser Phe Asp Lys Leu Asn Asn Tyr
    610                     615                     620

TTA ACT TTT AAC AAG TTC TGT TTG TCG TTG AAT CCT GTT GGT GCC AAC      1979
Leu Thr Phe Asn Lys Phe Cys Leu Ser Leu Asn Pro Val Gly Ala Asn
625                 630                     635                 640

TGT AAG TTT GAT GTT GCC GCC CGT ACA AGA ACC AAT GAG CAG GTT GTT      2027
Cys Lys Phe Asp Val Ala Ala Arg Thr Arg Thr Asn Glu Gln Val Val
                645                     650                 655

AGA AGT TTA TAT GTA ATG TAT GAA GAA GGA GAT AAC ATA GCG GGT GAC      2075
Arg Ser Leu Tyr Val Met Tyr Glu Glu Gly Asp Asn Ile Ala Gly Asp
            660                     665                     670

CGT CCT GAT AAT AGT GGT CTT CAC GAT TTG TCA GTG CTA CAC TTA GAT      2123
Arg Pro Asp Asn Ser Gly Leu His Asp Leu Ser Val Leu His Leu Asp
        675                     680                     685

TCC TGT ACA GAT TAC AAT ATA TAT GGT AGA ACT GGT GTT GGT ATT ATT      2171
Ser Cys Thr Asp Tyr Asn Ile Tyr Gly Arg Thr Gly Val Gly Ile Ile
    690                     695                     700

AGA CAA ACT AAC AGC ACA ATA TTT AGT GGC TTA TAT TAC ACA TCA CTA      2219
Arg Gln Thr Asn Ser Thr Ile Phe Ser Gly Leu Tyr Tyr Thr Ser Leu
705                 710                     715                 720

TCA GGT GAT TTG TTA GGT TTT AAA AAT GTT AGT GAT GGT GTC GTC TAT      2267
Ser Gly Asp Leu Leu Gly Phe Lys Asn Val Ser Asp Gly Val Val Tyr
                725                     730                 735

TCT GTA ACG CCA TGT GAT GTA AGC GCA CAA GCT GCT GTT ATT GAT GGT      2315
Ser Val Thr Pro Cys Asp Val Ser Ala Gln Ala Ala Val Ile Asp Gly
            740                     745                     750
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GCC ATA GTT GGA GCT ATG ACT TCC ATT AAT AGC GAA CTG TTA GGT CTA      2363
Ala Ile Val Gly Ala Met Thr Ser Ile Asn Ser Glu Leu Leu Gly Leu
        755                 760                 765

ACT CAT TGG ACA ACA ACA CCT AAT TTT TAT TAT TAC TCC ATA TAT AAT      2411
Thr His Trp Thr Thr Thr Pro Asn Phe Tyr Tyr Tyr Ser Ile Tyr Asn
    770                 775                 780

TAT ACA AGT GTG AGA ACT CGT GGC ACT GCA ATT GAT AGT AAC GAT GTT      2459
Tyr Thr Ser Val Arg Thr Arg Gly Thr Ala Ile Asp Ser Asn Asp Val
785                 790                 795                 800

GAT TGT GAA CCT ATC ATA ACC TAT TCT AAT ATA GGT GTT TGT AAA AAT      2507
Asp Cys Glu Pro Ile Ile Thr Tyr Ser Asn Ile Gly Val Cys Lys Asn
                805                 810                 815

GGA GCT TTG GTT TTT ATT AAC GTC ACA CAT TCT GAT GGA GAC GTT CAA      2555
Gly Ala Leu Val Phe Ile Asn Val Thr His Ser Asp Gly Asp Val Gln
            820                 825                 830

CCA ATT AGC ACC GGT AAT GTC ACG ATA CCT ACA AAT TTT ACC ATA TCT      2603
Pro Ile Ser Thr Gly Asn Val Thr Ile Pro Thr Asn Phe Thr Ile Ser
        835                 840                 845

GTG CAA GTT GAA TAC ATT CAG GTT TAC ACT ACA CCA GTG TCA ATA GAC      2651
Val Gln Val Glu Tyr Ile Gln Val Tyr Thr Thr Pro Val Ser Ile Asp
    850                 855                 860

TGT GCA AGA TAC GTT TGC AAT GGT AAC CCT AGA TGC AAT AAA TTG TTA      2699
Cys Ala Arg Tyr Val Cys Asn Gly Asn Pro Arg Cys Asn Lys Leu Leu
865                 870                 875                 880

ACA CAA TAT GTT TCT GCA TGT CAA ACT ATT GAG CAA GCA CTT GCA ATG      2747
Thr Gln Tyr Val Ser Ala Cys Gln Thr Ile Glu Gln Ala Leu Ala Met
                885                 890                 895

GGT GCC AGA CTT GAA AAC ATG GAG ATT GAT TCC ATG TTG TTT GTT TCG      2795
Gly Ala Arg Leu Glu Asn Met Glu Ile Asp Ser Met Leu Phe Val Ser
            900                 905                 910

GAA AAT GCC CTT AAA TTG GCG TCT GTT GAA GCA TTC AAT AGT ACG GAA      2843
Glu Asn Ala Leu Lys Leu Ala Ser Val Glu Ala Phe Asn Ser Thr Glu
        915                 920                 925

ACT CTA GAT CCT ATT TAC AAA GAA TGG CCC AAT ATT GGT GGT TCT TGG      2891
Thr Leu Asp Pro Ile Tyr Lys Glu Trp Pro Asn Ile Gly Gly Ser Trp
    930                 935                 940

CTA GGA GGT TTA AAA GAT ATA TTG CCA TCT CAT AAT AGC AAA CGT AAG      2939
Leu Gly Gly Leu Lys Asp Ile Leu Pro Ser His Asn Ser Lys Arg Lys
945                 950                 955                 960

TAC CGT TCA GCT ATA GAA GAC TTG CTT TTT GAT AAG GTT GTA ACA TCT      2987
Tyr Arg Ser Ala Ile Glu Asp Leu Leu Phe Asp Lys Val Val Thr Ser
                965                 970                 975
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GGC TTA GGT ACA GTT GAT GAA GAT TAT AAG CGT TGT ACA GGT GGT TAT      3035
Gly Leu Gly Thr Val Asp Glu Asp Tyr Lys Arg Cys Thr Gly Gly Tyr
            980                 985                 990

GAT ATA GCT GAC TTA GTG TGT GCA CAA TAT TAT AAT GGC ATC ATG GTG      3083
Asp Ile Ala Asp Leu Val Cys Ala Gln Tyr Tyr Asn Gly Ile Met Val
        995                 1000                1005

CTA CCT GGT GTA GCT AAT GAT GAC AAG ATG GCT ATG TAC ACT GCA TCT      3131
Leu Pro Gly Val Ala Asn Asp Asp Lys Met Ala Met Tyr Thr Ala Ser
    1010                1015                1020

CTT GCA GGT GGT ATA ACA TTA GGT GCA CTA GGT GGT GGC GCC GTG GCT      3179
Leu Ala Gly Gly Ile Thr Leu Gly Ala Leu Gly Gly Gly Ala Val Ala
1025                1030                1035                1040

ATA CCT TTT GCA GTA GCA GTT CAG GCT AGA CTT AAT TAT GTT GCT CTA      3227
Ile Pro Phe Ala Val Ala Val Gln Ala Arg Leu Asn Tyr Val Ala Leu
                1045                1050                1055

CAA ACT GAT GTA TTG AAC AAA AAC CAA CAG ATC CTG GCT AAT GCT TTC      3275
Gln Thr Asp Val Leu Asn Lys Asn Gln Gln Ile Leu Ala Asn Ala Phe
            1060                1065                1070

AAC CAA GCT ATT GGT AAC ATT ACA CAG GCA TTT GGT AAG GTT AAT GAC      3323
Asn Gln Ala Ile Gly Asn Ile Thr Gln Ala Phe Gly Lys Val Asn Asp
        1075                1080                1085

GCA ATA CAT CAA ACA TCA CAA GGT CTT GCC ACT GTT GCT AAA GCA TTG      3371
Ala Ile His Gln Thr Ser Gln Gly Leu Ala Thr Val Ala Lys Ala Leu
    1090                1095                1100

GCA AAA GTG CAA GAT GTT GTT AAC ACA CAA GGT CAA GCT TTA AGC CAC      3419
Ala Lys Val Gln Asp Val Val Asn Thr Gln Gly Gln Ala Leu Ser His
1105                1110                1115                1120

CTA ACA GTA CAA TTG CAA AAC AAT TTT CAA GCC ATT AGT AGT TCC ATT      3467
Leu Thr Val Gln Leu Gln Asn Asn Phe Gln Ala Ile Ser Ser Ser Ile
                1125                1130                1135

AGT GAC ATT TAC AAC AGG CTT GAT GAA TTG AGT GCT GAT GCA CAA GTT      3515
Ser Asp Ile Tyr Asn Arg Leu Asp Glu Leu Ser Ala Asp Ala Gln Val
            1140                1145                1150

GAC AGG CTT ATT ACA GGA AGA CTT ACA GCA CTT AAT GCA TTT GTG TCT      3563
Asp Arg Leu Ile Thr Gly Arg Leu Thr Ala Leu Asn Ala Phe Val Ser
        1155                1160                1165

CAG ACT TTA ACC AGA CAA GCA GAG GTT AGG GCT AGT AGA CAA CTT GCT      3611
Gln Thr Leu Thr Arg Gln Ala Glu Val Arg Ala Ser Arg Gln Leu Ala
    1170                1175                1180

AAA GAC AAA GTT AAT GAA TGC GTT AGG TCT CAA TCC CAG AGA TTT GGA      3659
Lys Asp Lys Val Asn Glu Cys Val Arg Ser Gln Ser Gln Arg Phe Gly
1185                1190                1195                1200
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TTC TGT GGT AAT GGT ACA CAT TTG TTT TCA CTT GCA AAT GCA GCA CCA      3707
Phe Cys Gly Asn Gly Thr His Leu Phe Ser Leu Ala Asn Ala Ala Pro
                1205                1210                1215

AAT GGC ATG ATT TTC TTT CAC ACA GTG CTA TTA CCA ACA GCT TAT GAA      3755
Asn Gly Met Ile Phe Phe His Thr Val Leu Leu Pro Thr Ala Tyr Glu
            1220                1225                1230

ACT GTG ACG GCC TGG TCA GGT ATT TGT GCA TCA GAT GGC GAT CGC ACT      3803
Thr Val Thr Ala Trp Ser Gly Ile Cys Ala Ser Asp Gly Asp Arg Thr
        1235                1240                1245

TTT GGA CTT GTT GTT AAG GAT GTT CAG CTG ACG CTA TTT CGC AAT TTA      3851
Phe Gly Leu Val Val Lys Asp Val Gln Leu Thr Leu Phe Arg Asn Leu
    1250                1255                1260

GAT GAC AAA TTC TAT TTG ACT CCC AGA ACT ATG TAT CAG CCT AGA GTT      3899
Asp Asp Lys Phe Tyr Leu Thr Pro Arg Thr Met Tyr Gln Pro Arg Val
1265                1270                1275                1280

GCA ACT AGT TCT GAT TTT GTT CAA ATA GAA GGT TGT GAT GTG TTG TTT      3947
Ala Thr Ser Ser Asp Phe Val Gln Ile Glu Gly Cys Asp Val Leu Phe
                1285                1290                1295

GTC AAT GCA ACT GTA ATT GAC TTG CCT AGT ATC ATA CCT GAC TAT ATT      3995
Val Asn Ala Thr Val Ile Asp Leu Pro Ser Ile Ile Pro Asp Tyr Ile
            1300                1305                1310

GAT ATT AAT CAA ACT GTT CAG GAT ATA TTA GAA AAT TTT AGA CCA AAT      4043
Asp Ile Asn Gln Thr Val Gln Asp Ile Leu Glu Asn Phe Arg Pro Asn
        1315                1320                1325

TGG ACT GTA CCT GAG TTG ACA CTT GAC ATT TTC AAC GCA ACC TAC TTA      4091
Trp Thr Val Pro Glu Leu Thr Leu Asp Ile Phe Asn Ala Thr Tyr Leu
    1330                1335                1340

AAC CTG ACT GGT GAA ATT AAT GAC TTA GAA TTT AGG TCG GAA AAG TTA      4139
Asn Leu Thr Gly Glu Ile Asn Asp Leu Glu Phe Arg Ser Glu Lys Leu
1345                1350                1355                1360

CAT AAC ACC ACA GTA GAA CTT GCT GTT CTC ATT GAT AAT ATT AAT AAC      4187
His Asn Thr Thr Val Glu Leu Ala Val Leu Ile Asp Asn Ile Asn Asn
                1365                1370                1375

ACA TTA GTC AAT CTT GAA TGG CTC AAT AGA ATT GAA ACT TAT GTA AAA      4235
Thr Leu Val Asn Leu Glu Trp Leu Asn Arg Ile Glu Thr Tyr Val Lys
            1380                1385                1390

TGG CCT TGG TAT GTG TGG CTA CTA ATT GGA TTA GTA GTA ATA TTC TGC      4283
Trp Pro Trp Tyr Val Trp Leu Leu Ile Gly Leu Val Val Ile Phe Cys
        1395                1400                1405

ATA CCA TTA CTG CTA TTT TGC TGT TGT AGT ACA GGT TGC TGT GGA TGC      4331
Ile Pro Leu Leu Leu Phe Cys Cys Cys Ser Thr Gly Cys Cys Gly Cys
    1410                1415                1420
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ATA GGT TGC TTA GGA AGT TGT TGT CAC TCT ATG TGT AGT AGA AGA CAA      4379
Ile Gly Cys Leu Gly Ser Cys Cys His Ser Met Cys Ser Arg Arg Gln
1425                1430                1435                1440

TTT GAA AGT TAT GAA CCA ACC GAA AAA GTG CAC GTC CAC TAAATTCAAA       4428
Phe Glu Ser Tyr Glu Pro Thr Glu Lys Val His Val His
                1445                1450

ACTAATA                                                              4435
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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1453 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Canine corona virus
(B) STRAIN: CCV-C54

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..1453
(D) OTHER INFORMATION: /label= CCV-C54_spike

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Ile Val Leu Thr Leu Cys Leu Leu Leu Phe Ser Tyr Asn Ser Val
1               5                   10                  15

Ile Cys Thr Ser Asn Asn Asp Cys Val Gln Val Asn Val Thr Gln Leu
            20                  25                  30

Pro Gly Asn Glu Asn Ile Ile Lys Asp Phe Leu Phe Gln Asn Phe Lys
        35                  40                  45

Glu Glu Gly Ser Val Val Val Gly Gly Tyr Tyr Pro Thr Glu Val Trp
    50                  55                  60

Tyr Asn Cys Ser Arg Thr Ala Thr Thr Thr Ala Tyr His Tyr Phe Ser
65                  70                  75                  80

Asn Ile His Ala Phe Tyr Phe Asp Met Glu Ala Met Ala Asn Ser Thr
                85                  90                  95

Gly Asn Ala Arg Gly Lys Pro Leu Leu Val His Val His Gly Ser Pro
            100                 105                 110
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Val Ser Ile Ile Val Tyr Ile Ser Ala Tyr Arg Asp Asp Val Gln Asn
        115                     120                     125

Arg Pro Leu Leu Lys His Gly Leu Leu Cys Ile Thr Lys Asn Ser Thr
    130                     135                     140

Ile Asp Tyr Asn Ser Phe Thr Ser Ala Gln Trp Arg Asp Ile Cys Leu
145                     150                     155                 160

Gly Thr Asp Arg Lys Ile Pro Phe Ser Val Val Pro Thr Asp Asn Gly
                165                     170                     175

Thr Lys Leu Phe Gly Leu Glu Trp Thr Asp Asp Tyr Val Thr Ala Tyr
            180                     185                     190

Ile Ser Asp Asp Ser His Arg Leu Asn Ile Asn Thr Asn Trp Phe Asn
        195                     200                     205

Asn Val Thr Ile Leu Tyr Ser Arg Ser Ser Thr Ala Thr Trp Gln Lys
    210                     215                     220

Ser Ala Ala Tyr Val Tyr Gln Gly Val Ser Asn Phe Thr Tyr Tyr Lys
225                     230                     235                 240

Leu Asn Asn Thr Asn Gly Leu Lys Ser Tyr Glu Leu Cys Glu Asp Tyr
                245                     250                     255

Glu Tyr Cys Thr Gly Tyr Ala Thr Asn Val Phe Ala Pro Thr Ser Gly
            260                     265                     270

Gly Tyr Ile Pro Asp Gly Phe Ser Phe Asn Asn Trp Phe Met Leu Thr
        275                     280                     285

Asn Ser Ser Thr Phe Val Ser Gly Arg Phe Val Thr Asn Gln Pro Leu
    290                     295                     300

Leu Val Asn Cys Leu Val Pro Val Pro Ser Phe Gly Val Ala Ala Gln
305                     310                     315                 320

Glu Phe Cys Phe Glu Gly Ala Gln Phe Ser Gln Cys Asn Gly Val Ser
                325                     330                     335

Leu Asn Asn Thr Val Asp Val Ile Arg Phe Asn Leu Asn Phe Thr Thr
            340                     345                     350

Asn Val Gln Ser Gly Met Gly Ala Thr Val Phe Ser Leu Asn Thr Thr
        355                     360                     365

Gly Gly Val Ile Leu Glu Ile Ser Cys Tyr Asn Asp Thr Val Ser Glu
    370                     375                     380

Ser Ser Phe Tyr Ser Tyr Gly Glu Ile Pro Phe Gly Val Thr Asp Gly
385                     390                     395                 400

Pro Arg Tyr Cys Tyr Val Leu Tyr Asn Gly Thr Ala Leu Lys Tyr Leu
                405                     410                     415
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Gly Thr Leu Pro Pro Ser Val Lys Glu Ile Ala Ile Ser Lys Trp Gly
            420                 425                 430

His Phe Tyr Ile Asn Gly Tyr Asn Phe Phe Ser Thr Phe Pro Ile Asp
        435                 440                 445

Cys Ile Ser Phe Asn Leu Thr Thr Gly Asp Ser Gly Ala Phe Trp Thr
    450                 455                 460

Ile Ala Tyr Thr Ser Tyr Thr Glu Ala Leu Val Gln Val Glu Asn Thr
465                 470                 475                 480

Ala Ile Lys Lys Val Thr Tyr Cys Asn Ser His Ile Asn Asn Ile Lys
                485                 490                 495

Cys Ser Gln Leu Thr Ala Asn Leu Gln Asn Gly Phe Tyr Pro Val Ala
            500                 505                 510

Ser Ser Glu Val Gly Leu Val Asn Lys Ser Val Val Leu Leu Pro Ser
        515                 520                 525

Phe Tyr Ser His Thr Ser Val Asn Ile Thr Ile Asp Leu Gly Met Lys
    530                 535                 540

Arg Ser Gly Tyr Gly Gln Pro Ile Ala Ser Thr Leu Ser Asn Ile Thr
545                 550                 555                 560

Leu Pro Met Gln Asp Asn Asn Thr Asp Val Tyr Cys Ile Arg Ser Asn
                565                 570                 575

Gln Phe Ser Val Tyr Val His Ser Thr Cys Lys Ser Ser Leu Trp Asp
            580                 585                 590

Asn Ile Phe Asn Ser Asp Cys Thr Asp Val Leu His Ala Thr Ala Val
        595                 600                 605

Ile Lys Thr Gly Thr Cys Pro Phe Ser Phe Asp Lys Leu Asn Asn Tyr
    610                 615                 620

Leu Thr Phe Asn Lys Phe Cys Leu Ser Leu Asn Pro Val Gly Ala Asn
625                 630                 635                 640

Cys Lys Phe Asp Val Ala Ala Arg Thr Arg Thr Asn Glu Gln Val Val
                645                 650                 655

Arg Ser Leu Tyr Val Met Tyr Glu Glu Gly Asp Asn Ile Ala Gly Asp
            660                 665                 670

Arg Pro Asp Asn Ser Gly Leu His Asp Leu Ser Val Leu His Leu Asp
        675                 680                 685

Ser Cys Thr Asp Tyr Asn Ile Tyr Gly Arg Thr Gly Val Gly Ile Ile
    690                 695                 700
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Arg Gln Thr Asn Ser Thr Ile Phe Ser Gly Leu Tyr Tyr Thr Ser Leu
705                 710                 715                 720

Ser Gly Asp Leu Leu Gly Phe Lys Asn Val Ser Asp Gly Val Val Tyr
                725                 730                 735

Ser Val Thr Pro Cys Asp Val Ser Ala Gln Ala Ala Val Ile Asp Gly
            740                 745                 750

Ala Ile Val Gly Ala Met Thr Ser Ile Asn Ser Glu Leu Leu Gly Leu
        755                 760                 765

Thr His Trp Thr Thr Thr Pro Asn Phe Tyr Tyr Tyr Ser Ile Tyr Asn
    770                 775                 780

Tyr Thr Ser Val Arg Thr Arg Gly Thr Ala Ile Asp Ser Asn Asp Val
785                 790                 795                 800

Asp Cys Glu Pro Ile Ile Thr Tyr Ser Asn Ile Gly Val Cys Lys Asn
                805                 810                 815

Gly Ala Leu Val Phe Ile Asn Val Thr His Ser Asp Gly Asp Val Gln
            820                 825                 830

Pro Ile Ser Thr Gly Asn Val Thr Ile Pro Thr Asn Phe Thr Ile Ser
        835                 840                 845

Val Gln Val Glu Tyr Ile Gln Val Tyr Thr Thr Pro Val Ser Ile Asp
    850                 855                 860

Cys Ala Arg Tyr Val Cys Asn Gly Asn Pro Arg Cys Asn Lys Leu Leu
865                 870                 875                 880

Thr Gln Tyr Val Ser Ala Cys Gln Thr Ile Glu Gln Ala Leu Ala Met
                885                 890                 895

Gly Ala Arg Leu Glu Asn Met Glu Ile Asp Ser Met Leu Phe Val Ser
            900                 905                 910

Glu Asn Ala Leu Lys Leu Ala Ser Val Glu Ala Phe Asn Ser Thr Glu
        915                 920                 925

Thr Leu Asp Pro Ile Tyr Lys Glu Trp Pro Asn Ile Gly Gly Ser Trp
    930                 935                 940

Leu Gly Gly Leu Lys Asp Ile Leu Pro Ser His Asn Ser Lys Arg Lys
945                 950                 955                 960

Tyr Arg Ser Ala Ile Glu Asp Leu Leu Phe Asp Lys Val Val Thr Ser
                965                 970                 975

Gly Leu Gly Thr Val Asp Glu Asp Tyr Lys Arg Cys Thr Gly Gly Tyr
            980                 985                 990

Asp Ile Ala Asp Leu Val Cys Ala Gln Tyr Tyr Asn Gly Ile Met Val
        995                 1000                1005
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Leu Pro Gly Val Ala Asn Asp Asp Lys Met Ala Met Tyr Thr Ala Ser
1010 1015 1020

Leu Ala Gly Gly Ile Thr Leu Gly Ala Leu Gly Gly Gly Ala Val Ala
1025 1030 1035 1040

Ile Pro Phe Ala Val Ala Val Gln Ala Arg Leu Asn Tyr Val Ala Leu
1045 1050 1055

Gln Thr Asp Val Leu Asn Lys Asn Gln Gln Ile Leu Ala Asn Ala Phe
1060 1065 1070

Asn Gln Ala Ile Gly Asn Ile Thr Gln Ala Phe Gly Lys Val Asn Asp
1075 1080 1085

Ala Ile His Gln Thr Ser Gln Gly Leu Ala Thr Val Ala Lys Ala Leu
1090 1095 1100

Ala Lys Val Gln Asp Val Val Asn Thr Gln Gly Gln Ala Leu Ser His
1105 1110 1115 1120

Leu Thr Val Gln Leu Gln Asn Asn Phe Gln Ala Ile Ser Ser Ser Ile
1125 1130 1135

Ser Asp Ile Tyr Asn Arg Leu Asp Glu Leu Ser Ala Asp Ala Gln Val
1140 1145 1150

Asp Arg Leu Ile Thr Gly Arg Leu Thr Ala Leu Asn Ala Phe Val Ser
1155 1160 1165

Gln Thr Leu Thr Arg Gln Ala Glu Val Arg Ala Ser Arg Gln Leu Ala
1170 1175 1180

Lys Asp Lys Val Asn Glu Cys Val Arg Ser Gln Ser Gln Arg Phe Gly
1185 1190 1195 1200

Phe Cys Gly Asn Gly Thr His Leu Phe Ser Leu Ala Asn Ala Ala Pro
1205 1210 1215

Asn Gly Met Ile Phe Phe His Thr Val Leu Leu Pro Thr Ala Tyr Glu
1220 1225 1230

Thr Val Thr Ala Trp Ser Gly Ile Cys Ala Ser Asp Gly Asp Arg Thr
1235 1240 1245

Phe Gly Leu Val Val Lys Asp Val Gln Leu Thr Leu Phe Arg Asn Leu
1250 1255 1260

Asp Asp Lys Phe Tyr Leu Thr Pro Arg Thr Met Tyr Gln Pro Arg Val
1265 1270 1275 1280

Ala Thr Ser Ser Asp Phe Val Gln Ile Glu Gly Cys Asp Val Leu Phe
1285 1290 1295

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Val Asn Ala Thr Val Ile Asp Leu Pro Ser Ile Ile Pro Asp Tyr Ile
            1300                1305                1310

Asp Ile Asn Gln Thr Val Gln Asp Ile Leu Glu Asn Phe Arg Pro Asn
        1315                1320                1325

Trp Thr Val Pro Glu Leu Thr Leu Asp Ile Phe Asn Ala Thr Tyr Leu
    1330                1335                1340

Asn Leu Thr Gly Glu Ile Asn Asp Leu Glu Phe Arg Ser Glu Lys Leu
1345                1350                1355                1360

His Asn Thr Thr Val Glu Leu Ala Val Leu Ile Asp Asn Ile Asn Asn
                1365                1370                1375

Thr Leu Val Asn Leu Glu Trp Leu Asn Arg Ile Glu Thr Tyr Val Lys
            1380                1385                1390

Trp Pro Trp Tyr Val Trp Leu Leu Ile Gly Leu Val Val Ile Phe Cys
        1395                1400                1405

Ile Pro Leu Leu Leu Phe Cys Cys Cys Ser Thr Gly Cys Cys Gly Cys
    1410                1415                1420

Ile Gly Cys Leu Gly Ser Cys Cys His Ser Met Cys Ser Arg Arg Gln
1425                1430                1435                1440

Phe Glu Ser Tyr Glu Pro Thr Glu Lys Val His Val His
                1445                1450
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Claims

1. A nucleic acid sequence encoding a polypeptide having one or more immunogenic determinants of a CCV spike protein.

2. A nucleic acid sequence according to claim 1, characterized in that the spike protein has an amino acid sequence shown in SEQ ID NO: 2, 4 or 6 or is a functional variant thereof.

3. A nucleic acid sequence according to claim 2, characterized in that the nucleic acid sequence contains at least part of the DNA sequence shown in SEQ ID NO: 1, 3 or 5.

4. A recombinant vector molecule comprising a nucleic acid sequence according to claims 1-3.

5. A recombinant vector molecule according to claim 4, characterized in that the nucleic acid sequence is operably linked to expression control sequences.

6. A recombinant vector virus harbouring the heterologous nucleic acid sequence according to claims 1-3.

7. A host cell transformed with a nucleic acid sequence according to claims 1-3 or with a recombinant vector molecule according to claim 4 or 5, or infected with a recombinant vector virus according to claim 6.

8. A process for the preparation of a polypeptide having one or more immunogenic determinants of a CCV spike protein which process comprises:
   (a) culturing host cells according to claim 7 under conditions in which the nucleic acid sequence is expressed, and
   (b) isolating the polypeptide from the culture.

9. A vaccine for the protection of dogs against CCV infection or disease, characterized in that it comprises a recombinant vector virus according to claim 6, a host cell according to claim 7, or a polypeptide

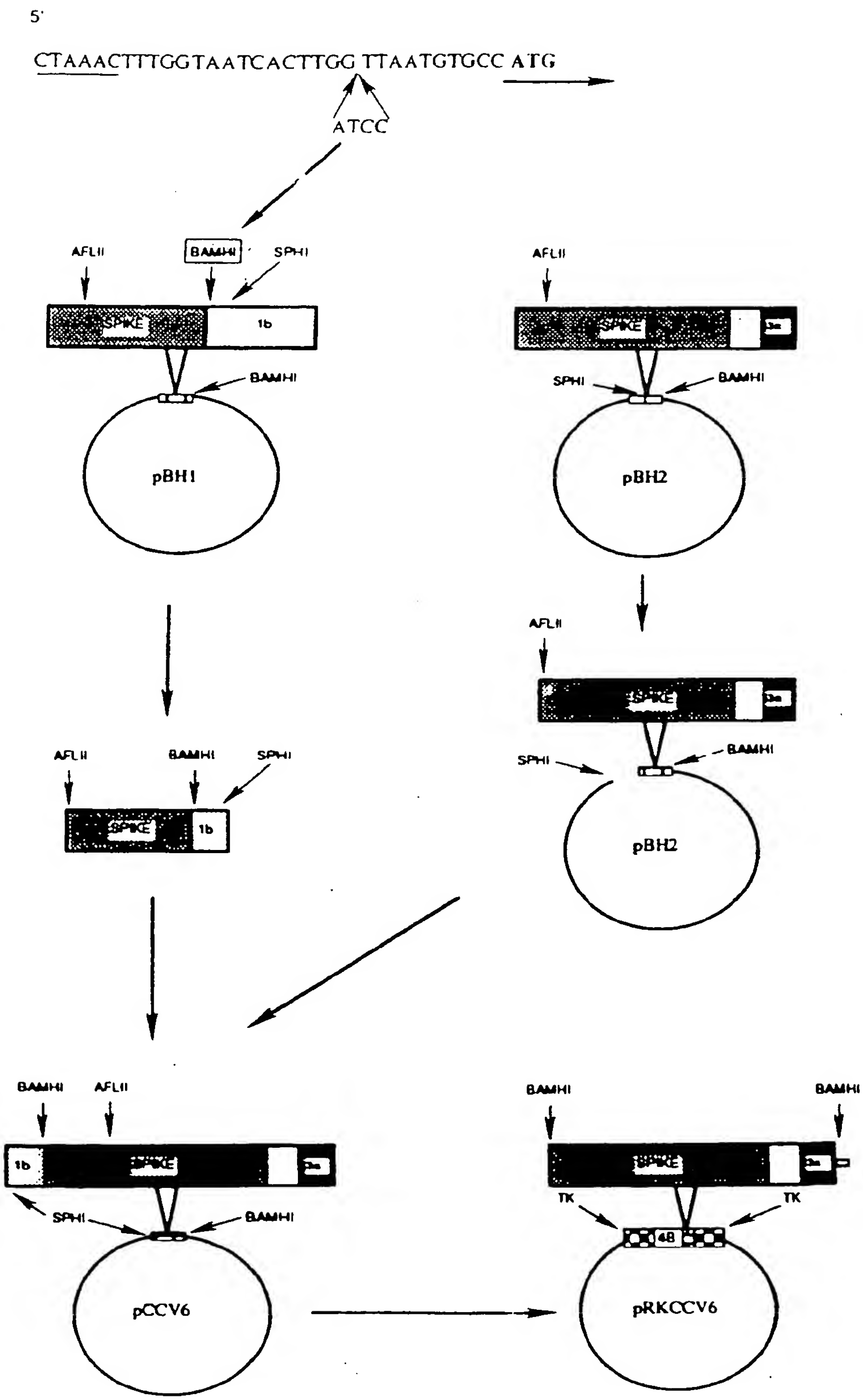
prepared by the process according to claim 8, together with an acceptable carrier.

10. A process for the preparation of a CCV vaccine comprising the steps of culturing an infected host cell according to claim 7, collecting recombinant vector virus material, and formulating the material to a pharmaceutical preparation with immunizing activity.

11. A process for the preparation of a CCV vaccine comprising formulating a polypeptide prepared to the process of claim 8 according to a pharmaceutical preparation with immunizing activity.

12. A process for the protection of dogs against CCV infection comprising administering a vaccine according to claim 9 to a dog.

Figure 1


5'
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ATCC
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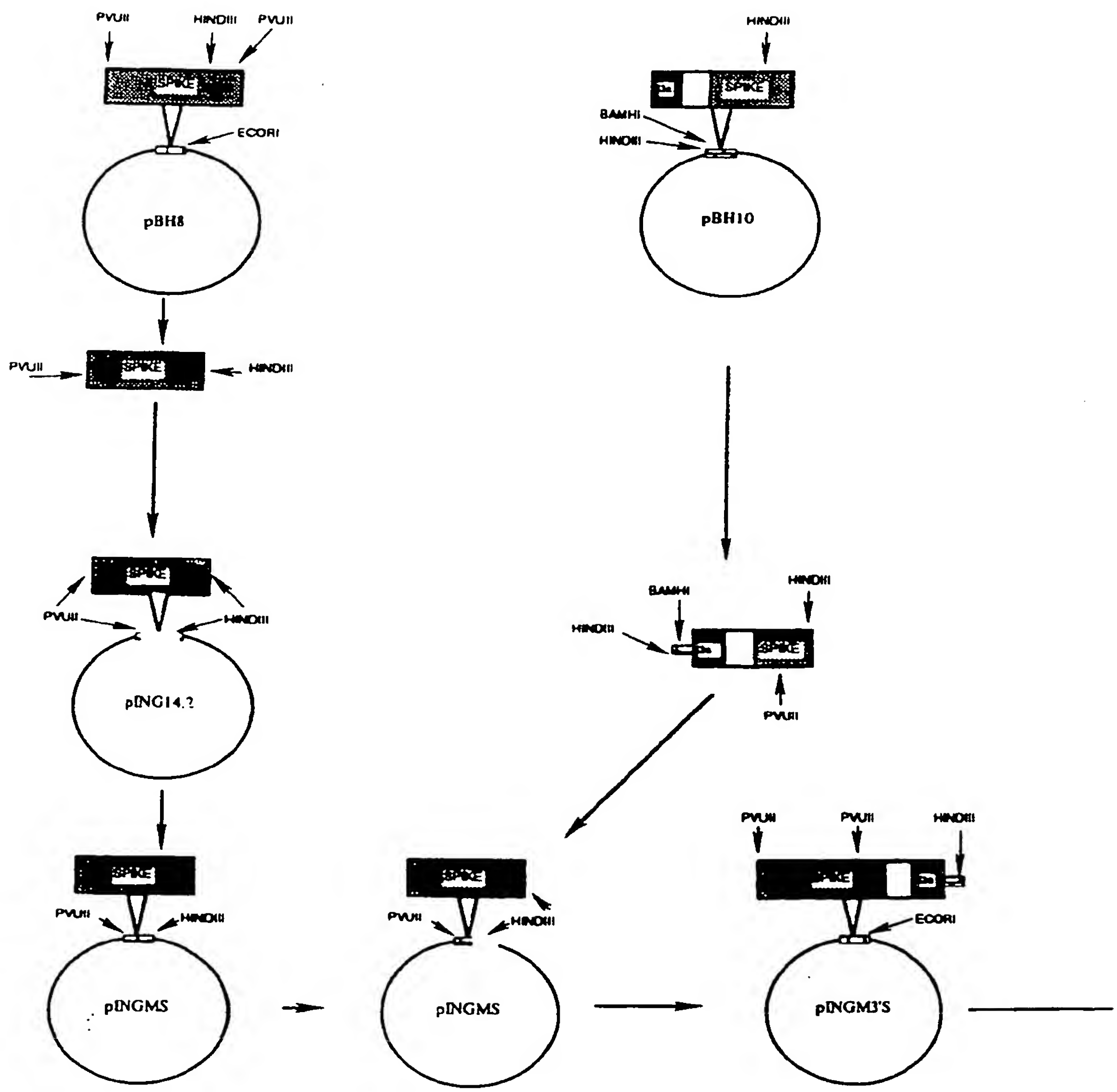
BAMHI
SPHI
SPIKE
1b
BAMHI
pBH1
AFLII
SPIKE
SPHI
BAMHI
pBH2
AFLII
BAMHI
SPHI
SPIKE
1b
AFLII
SPIKE
SPHI
BAMHI
pBH2
BAMHI
AFLII
1b
SPIKE
SPHI
BAMHI
pCCV6
BAMHI
BAMHI
SPIKE
TK
TK
48
pRKCCV6

Figure 2a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HINDIII
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SPIKE
ECORI
pBH8
PVUII
SPIKE
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SPIKE
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pING14.2
SPIKE
PVUII
HINDIII
pINGMS
HINDIII
SPIKE
BAMHI
HINDIII
pBH10
BAMHI
HINDIII
HINDIII
SPIKE
PVUII
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HINDIII
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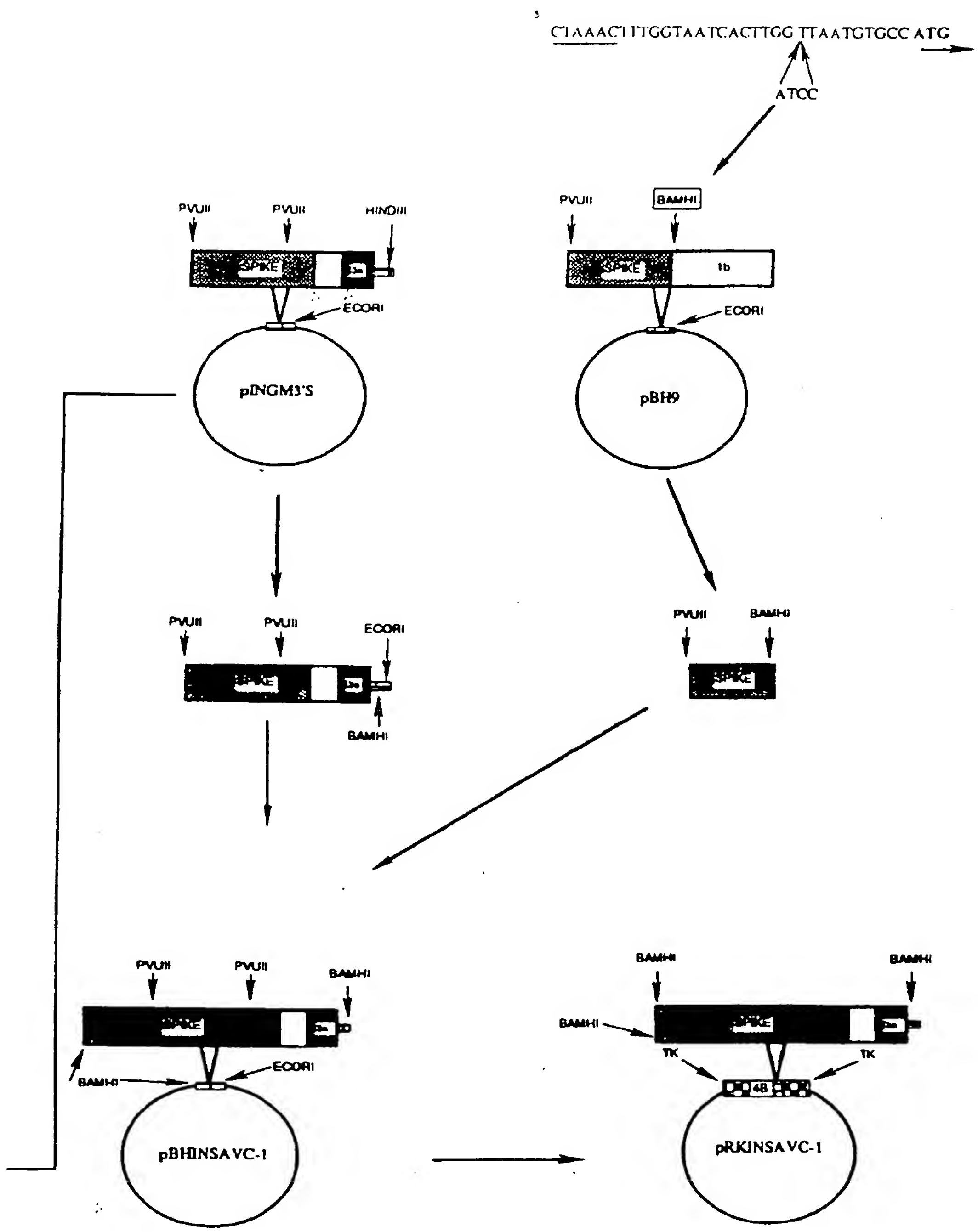
PVUII
PVUII
HINDIII
SPIKE
ECORI
pINGM3'S

Figure 2b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ATCC
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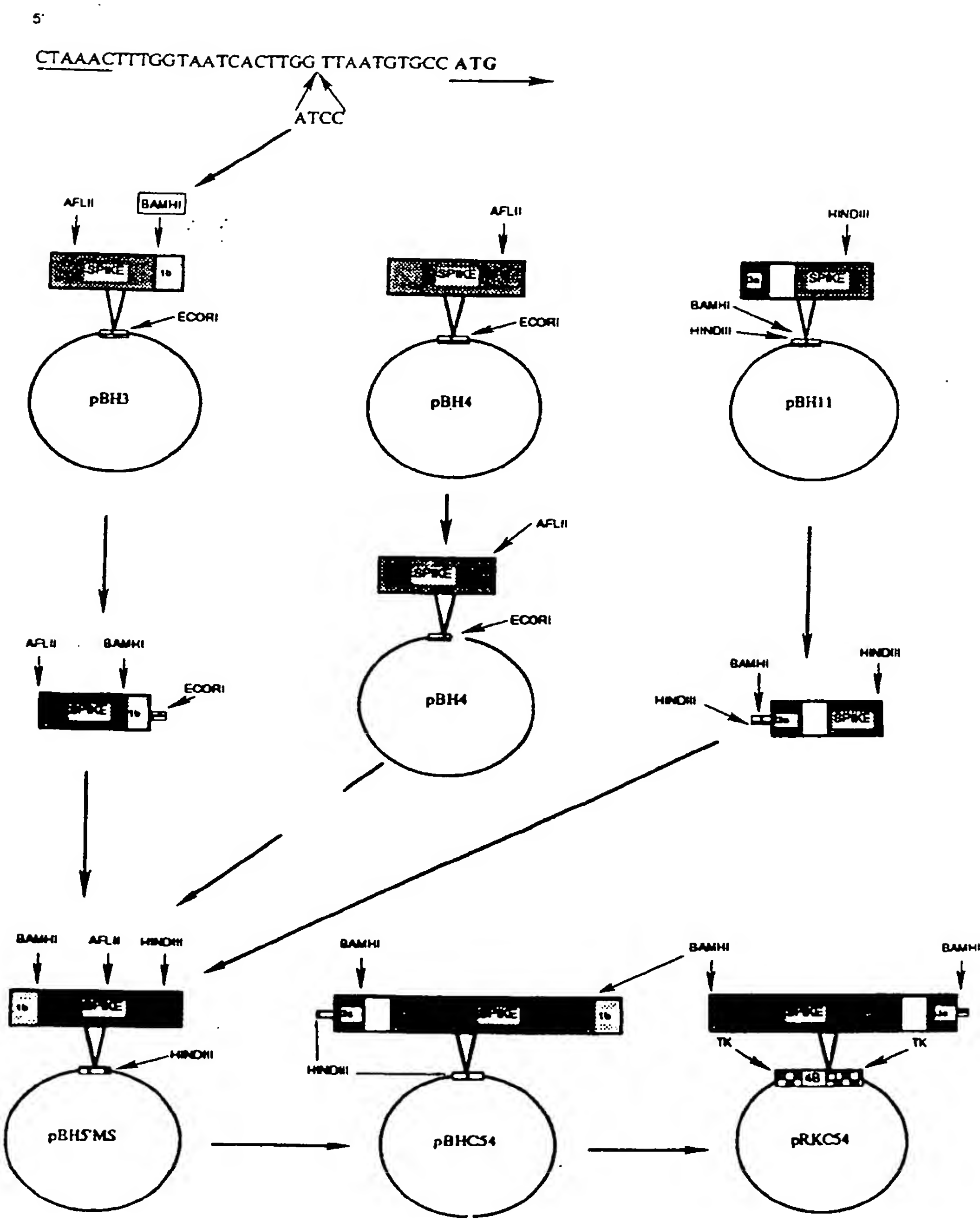
HINDIII
SPIKE
ECORI
pINGM3'S
BAMHI
pBH9
pBHINSAVC-1
TK
pRKINSAVC-1

Figure 3


5'
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ATCC
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BAMHI
SPIKE
ECORI
pBH3
pBH4
HINDIII
pBH11
BAMHI
pBH5'MS
pBHC54
TK
pRKC54

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 92 20 1136

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	EP-A-0 295 057 (NORDEN LABORATORIES, INC.) * whole document, in particular page 3, lines 50-51, page 5 lines 51-56 * ---	1-12	C12N15/50 A61K39/215 G01N33/569 C07K15/00
Y	EP-A-0 376 744 (CALIFORNIA BIOTECHNOLOGY, INC) * whole document * ---	1-12	
A	EP-A-0 138 242 (DUPHAR INTERNATIONAL RESEARCH B.V.) * whole document * ---	1,4,7, 9-10	
Y	JOURNAL OF CLINICAL MICROBIOLOGY vol. 29, no. 1, January 1991, US I. BAE ET AL.: 'Differentiation of transmissible gastroenteritis virus from porcine respiratory coronavirus and other antigenically related coronaviruses by using cDNA probes specific for the 5' region of the S glycoprotein gene' * whole article * ---	1-5,7-12	
Y	EP-A-0 278 541 (DUPHAR INTERNATIONAL RESEARCH B.V.) * whole document * ---	1-5,7-12	TECHNICAL FIELDS SEARCHED (Int. Cl.5) C12N A61K C07K
A	EP-A-0 344 872 (AMERICAN HOME PRODUCTS CORPORATION) * whole document * ---	1	
P,X, D	WO-A-9 111 525 (THE UNIVERSITY COURT OF THE UNIVERSITY OF GLASGOW) * whole document * -----	1,4,6-12	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	07 JULY 1992	JULIA P.

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& : member of the same patent family, corresponding document